THE CALCIUM REQUIREMENTS OF FIVE PRE-SCHOOL GIRLS [1]

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ONE FIGURE

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In 1922 Sherman and Hawley reported that a daily intake of 1 gm. of calcium was necessary to induce optimum storage of this element in children from 3 to 13 years of age. Since that time data on calcium retentions at various levels of intake have been reported for numerous children ranging in age from 3 to 6 years inclusive. Taken as a whole, the observed data afford some evidence that the retentions of calcium on daily intakes of 1 gm. or more of calcium were higher than on intakes of less than 1 gm. However, for the seventy children for whom individual metabolic data are given in the literature (Sawyer, Bauman and Stevens, '18; Willard and Blunt, '27; Wang, Kaucher and Frank, '28; Burton, '29; Wang, Kern and Kaucher, '30; Porter-Levin, '32–'33 and '33–'34; Hunscher et al., '33; Petrunkina, '34; Daniels et al., '34, '35; Hunscher, Hummel and Macy, '36–'37; Bonner et al., '38), great inter- and intra-individual variations in the retentions of calcium were observed.

[1] Portions of these data were presented before the American Institute of Nutrition, Memphis, Tennessee, April, 1937, and before the American Home Economics Association, Kansas City, Missouri, June, 1937.

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From such inconsistent data it would be hazardous to draw conclusions concerning the minimum quantity of calcium required to insure maximum retention or to predict the quantity of calcium which might be retained on a given level of intake.

The nutritional status of the individual, in regard to the saturation of the bones with calcium, has been suggested as one of the reasons for the variability in retentions among children. Hummel et al., ('37) have reviewed the published evidence for this suggestion and have accepted it as the explanation for the great differences in calcium retentions which they observed in pregnant women. Proof of the contention that the previous regimen, in regard to calcium, influences the amount of calcium retained subsequently has been given by Fairbanks and Mitchell ('36). These authors found that the amount of calcium retained by rats during a given period varied inversely with the level of calcium in the ration fed previous to that period. They concluded that ". . . . the rate of calcium retention by growing animals under conditions of adequate nutrition measures the requirement of calcium only when the calcium stores have been saturated by appropriate pre-feeding." These findings point definitely to the necessity for careful preparation of all prospective subjects to be used in studies on calcium requirement. "Otherwise," in the words of the above investigators, "the observed calcium retentions will be greater than the day-to-day requirements for calcium."

Surprising fluctuations in the amount of calcium retained during several consecutive metabolic periods by growing children on a constant intake of calcium have been reported by Porter-Levin ('33–'34), by Hunscher, Hummel and Macy ('36–'37), and by Bonner et al. ('38). On analyzing her data, Porter-Levin ('33–'34) found that from 15 to 21 consecutive days were required to cover the range of variations in retentions of her subjects. In view of this fact, it would seem that much of the reported data based on short collection periods must necessarily be re-evaluated.

The purpose of the present study was to determine the maximum rate of calcium retention in well-nourished children (this measures the day-to-day calcium requirements for growth) and also to find the minimum daily intake of dietary calcium which would support maximum retention. At the beginning of the experiment, an attempt was made to saturate the body tissues with calcium by feeding generous quantities over a comparatively long period of time. It was hoped that this procedure would tend to minimize the influence of the previous regimens on the subsequent calcium metabolism of the subjects. The experimental periods which followed this

TABLE 1

The ages, weights and heights of the subjects and the comparisons of these with Grandprey's ('33) standards

SUBJECT	AGE[1]		WEIGHT[1]	HEIGHT[1]	LOCUS OF MEASUREMENTS		
	Years	Months			Weight—Age	Height—Age	Weight—height
			kg.	*cm.*	%	%	%
B	3	9	14.1	93.5	65	25	Above 90
M	3	11	13.4	98.5	30	60	30
J	5	2	17.0	100.2	60	25	Above 90
P	5	8	17.4	110.5	50	70	65
S	5	11	18.2	105.4	75	10	Above 90

[1] Taken at seventeenth week of experiment.

period of preparation were sufficiently long to show the fluctuations characteristic of the individual and to obtain a reliable mean value for the child's requirement.

EXPERIMENTAL

Subjects. Five healthy, pre-school girls, whose ages, heights, weights and the comparisons thereof with Grandprey's ('33) modifications of Woodbury's standards are given in table 1, were selected for the study.

The basal diet. The basal dietary was generous in all of the known necessary nutrients except calcium. Seven different groups of foods were selected (one for each day of the week), and these were fed weekly throughout the 32 weeks of the experiment. These foods are listed in table 2. A smaller

TABLE 3

Constituents of the basal diet for each day of the week

WEDNESDAY		THURSDAY		FRIDAY		SATURDAY		SUNDAY		MONDAY		TUESDAY	
Article of food	Weight	Article of food	Weight	Article of food	Weight	Article of food	Weight	Article of food	Weight	Article of food	Weight	Article of food	Weight
	gm.		gm.		gm.		gm.		gm.		gm.		gm.
Apple	100	Pears	50	Peaches	50	Banana	70	Grapefruit	40	Prunes	25	Apricots	20
Lemon juice	45	Apple	75	Pineapple	50	Apple	75	Grapefruit juice	10	Pineapple	25	Prunes	15
Fruit juices	100	Grapefruit	40	Prunes	25	Carrots	20	Pear	50	Banana	70	Pears	75
Peaches	50	Grapefruit juice	10	Tomato juice	100	Beans	30	Pear juice	10	Tomato juice	50	Beans (green)	20
Tomato juice	100	Carrots	10	Lettuce	10	Potato	75	Banana	50	Cabbage	25	Potato	100
Potato	50	Cabbage	25	Potato	50	Oatmeal	15	Potato	100	Potato	100	Farina	25
Oatmeal	10	Potato	50	Spaghetti	15	Beef	60	Sweet potato	75	Rice	20	Beef	60
Rice	10	Cornmeal	20	Rice	15	Egg white	68	Farina	20	Tapioca	10	Egg white	68
Spaghetti	15	Macaroni	15	Tuna fish	60	Egg yolk	16	Chicken	60	Beef	60	Egg yolk	16
Liver	60	Dried beef	40	Egg white	68			Chicken broth	100	Egg white	68		
Egg white	68	Egg white	68	Egg yolk	16			Egg white	68	Egg yolk	16		
Egg yolk	16	Egg yolk	16					Egg yolk	16				

quantity of vegetables than is generally recommended for children of this age was given in order that the diet might be low in calcium and accurately sampled for analytical purposes. Substances which were added to this diet, in order to compensate for the lack of vegetables or to make possible any necessary changes in the calcium and nitrogen intakes, are shown in table 3. In addition to these foods and concentrates, all of which were fed in definite daily quantities, each child ate ad libitum of bread (from which the crusts had been removed), butterfat, C.P. sugar and jelly in quantities sufficient to meet her individual caloric demands. A small variable portion of cornstarch was used in cooking. Redistilled water

TABLE 3

Daily supplements to the basal diet

PERIOD	CALCIUM AS MILK SOLIDS	CALCIUM AS di-CALCIUM PHOSPHATE	CASEIN	IRON AS FERRIC CHLORIDE	TIKI-TIKI	VITAMIN D AS COD LIVER OIL	ASCORBIC ACID
	mg.	*mg.*	*gm.*	*mg.*	*cc.*	*Int. units*	*mg.*
I	258	1466	10	15	5	360	100
II	258	....	10	15	..	360–1000[1]	100
III	516	....	3	15	5	1000	100
IV	774	....	3	15	5	1000	100

[1] At the twenty-first week the cod liver oil intake was increased from 1 teaspoonful to 3 daily.

was used for cooking and drinking purposes as well as for the rinsing of dishes.

The calcium level. As is indicated in table 3, the calcium was given as a supplement at four different levels during the experiment. For the first 15 weeks (period 1) generous amounts of di-calcium phosphate were fed. Following this interval, the calcium intake was decreased to approximately 370 mg. daily during the 8 weeks of period II. During periods III and IV, the level was raised successively to approximate averages of 615 and 880 mg., respectively.

Preparation of material for analysis. A 7-day metabolic period was selected. Carmine (350 mg.) was fed as a marker at the beginning of each weekly period. The total urinary and

fecal outputs were collected for each consecutive week with the exception of the eleventh, twelfth and thirteenth weeks.

In weighing the foods for each meal, a portion identical with that to be served was saved; all such samples for the week were combined for analysis. The basal food, the bread and the stools were prepared for analysis according to the method of Stearns ('28–'29). The materials were allowed to digest in hydrochloric acid (1 : 5 dilution) until they could be strained through a 1 mm.-pore Büchner funnel. The small amount of indigestible fiber in both foods and stools was ashed separately and added to the corresponding digest. The strained material was then made to volume, and aliquots were withdrawn by means of special pipettes which had been prepared for use by breaking the tip (in order to increase the diameter of the bore) and re-standardized. Aliquots of the week's collection of urine were analyzed separately. The dish washings, obtained by cleaning the dishes with a rubber policeman and redistilled water, were also analyzed for the calcium content. They were evaporated to a small volume and digested with an acid-digestion mixture (1 part HNO_3 to 1 part H_2SO_4). The digest then was made to volume.

Analysis for calcium. The samples were evaporated to dryness over a steam bath, ashed in a muffle furnace at 600°C. and treated for the removal of silicates according to Frear and Kahlenberg's method ('33). Calcium then was determined by McCrudden's method ('11–'12). Di-brom cresol purple was used as an indicator, and the calcium was titrated with a standard solution of potassium permanganate.

RESULTS

The data for the calcium intake and balance for each subject during the four periods are given in table 4. The weekly balances for the five subjects (expressed as the daily average), as well as the average of the five values, are shown graphically in figure 1.

Calcium metabolism at a level of 1815 mg. daily (period I). The daily intakes of calcium during this period varied from 1804 to 1835 mg. for the five children and averaged 1815 mg.

As seen in table 3, the calcium was derived chiefly from di-calcium phosphate (1466 mg. daily). An additional quantity of calcium (averaging 100 mg.) was supplied by the basal food, and 258 mg. were contributed by milk solids.[2] The latter was equivalent to approximately 220 cc. of fluid milk.

The calcium balance of the individual subjects varied considerably from week to week. This variation may have been due in part to the inadequacy of the carmine marker to insure a complete separation of the feces, especially since the feces contained large amounts of calcium. The individual average values for the retention of calcium during

TABLE 4

Daily calcium intake and retention for subjects

PERIOD		CALCIUM INTAKE (AVERAGE)	CALCIUM RETENTION					
Number	Weeks		Subject B	Subject M	Subject J	Subject P	Subject S	Average
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
I	11	1800	121	118	151	145	94	126
II	8	370	72	50	94	80	84	76
III	5	615	117	84	145	142	124	122
IV	4	880	108	85	118	154	94	112

the 10 weeks for which analyses were available were 121, 118, 151, 145 and 94, for subjects B, M, J, P and S, respectively, with a mean of 126 mg. daily.

Calcium metabolism at a level of 370 mg. daily (period II). During this period the total daily intake of calcium was approximately 370 mg. The decrease in the calcium intake from that of period I was effected by the removal of the di-calcium phosphate supplement from the regimen for that period. No other change was made in the diet. The quantity of calcium given during this period was considerably less than that amount recommended for children of this age. However, it was from two to four times greater than the average retention during the period of liberal intake of calcium, and maxi-

[2] Skim milk powder, the calcium content of the various lots of which ranged from 0.0123 to 0.0133 mg. per gram.

mum retention could have been effected by every one of the subjects if 50% of the calcium had been available (Leitch, '37). But in each of the five subjects the retention of calcium decreased. As is seen in table 4 and figure 1, the mean values for the calcium balances during period II were 72, 50, 94, 80 and 84 mg. daily for subjects B, M, J, P and S, respectively, with a total average of 76 mg.

Calcium metabolism at a level of 615 mg. daily (period III). The calcium intake during this period was increased over that in period II by the daily addition of a quantity of milk solids equivalent to 220 cc. of fluid milk to the diet of period II. It was desirable that the resultant level of intake would be slightly below (or barely equal to) the minimum amount required for maximum retention. The average retentions for each child were greater than during the previous period. The mean values were 117, 84, 145, 142 and 124 mg. for subjects B, M, J, P and S, respectively, with a total average of 122 mg. The values for subjects B, J and P were within 4% of the values obtained for period I. The value for subject B was considerably lower than and that for subject S was considerably higher than, the corresponding value for period I. The total averages for the two periods were almost identical.

Calcium metabolism at a level of 880 mg. daily (period IV). The calcium level during this period was obtained by adding milk solids equivalent to 220 cc. of fluid milk daily to the dietary fed during period III. The total milk consumption was approximately 660 cc. daily. This additional calcium was given in order to find whether or not it was possible to obtain a greater storage of calcium than was found during periods I and III. The values obtained for subjects B, M, J, P and S were 108, 85, 118, 154 and 94 mg., respectively, with an average of 112 mg. daily.

Fig. 1 The columns represent the average daily retention of calcium in milligrams for each week. The horizontal dotted lines represent the average daily retention for the various periods. The blank spaces indicate weeks during which analyses were not made, although the children were maintained on the diet. During the fifteenth week the children had a respiratory infection.

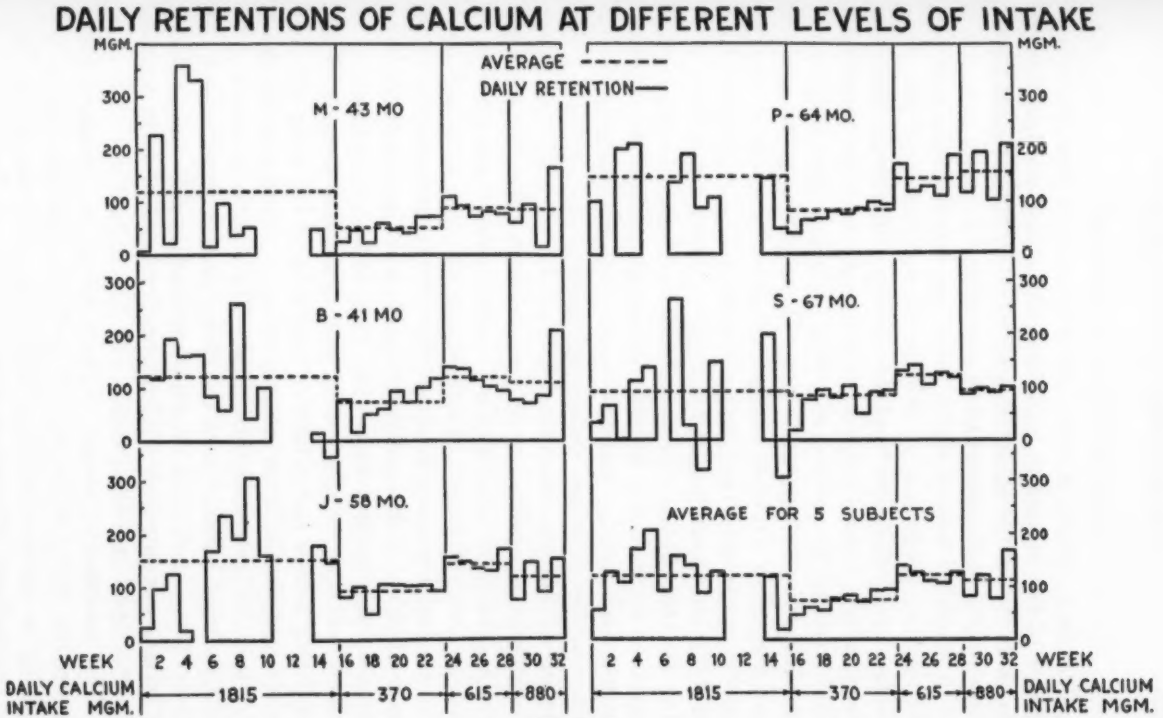
DAILY RETENTIONS OF CALCIUM AT DIFFERENT LEVELS OF INTAKE
MGM.
AVERAGE
DAILY RETENTION
M - 43 MO
P - 64 MO.
B - 41 MO
S - 67 MO.
J - 58 MO.
AVERAGE FOR 5 SUBJECTS
300
200
100
0
WEEK
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
DAILY CALCIUM INTAKE MGM.
1815
370
615
880

Figure 1

DISCUSSION AND CONCLUSIONS

The data presented above will be discussed from the standpoints of the maximum quantities of calcium retained by the subjects and of the minimum amount of dietary calcium necessary to induce retentions of this magnitude.

In selecting values which would be representative of the day-by-day accretions of calcium in the bodies of the individual subjects under optimal dietary conditions, the data from period I were not considered because of the possibility that some of the calcium stored at this time was for the replenishment of depleted stores. During subsequent periods the maximum average amount retained by a given subject during a given period was considered to be characteristic of that individual's growth needs. These retentions were 117, 84, 145, 154 and 124 mg. of calcium daily (averaging 125 mg.), or 7.8, 6.2, 8.3, 8.8 and 6.5 mg. per kilogram (with an average of 7.5), for subjects B, M, J, P and S, respectively. None of these actual values approaches the average daily figure of 344 mg. recommended by Leitch ('37) as being necessary for skeletal growth of children. But the estimate of Leitch is obviously too high. A few authors have observed similar low retentions of calcium in children who had been fed presumably generous quantities of calcium but apparently have attached little significance to them. These authors have emphasized the maximum quantity retained even though the value was the result of only one analytical period. It is probable that a lower figure, especially if it is the average of several metabolic periods, more nearly represents the daily needs of the well-nourished child. The results of the present study would indicate that the daily growth requirement for these pre-school girls was approximately 125 mg. with a standard deviation of ± 2.7 or, on the basis of body weight, 7.5 mg. $\pm$ 1.1 per kilogram.

The maximum average calcium retentions, ranging from 84 to 154 mg. daily, were attained on a calcium intake not greatly in excess of 600 mg. daily. On a 615 mg. intake, four of the subjects retained as much calcium as they did on the

higher level (i.e., 880 mg.). Subject P retained slightly more calcium at this latter level than she did during any other period. The quantity of dietary calcium which could insure a retention of 154 mg. may be estimated on the basis of her individual ability to utilize dietary calcium. This figure would be approximately 665 mg. daily or 38 mg. per kilogram. For the remaining subjects, i.e., B, M, J, and S, the dietary requirements, calculated on the basis of weight, were 41, 44, 35 and 32 mg. per kilogram, respectively. None of these values fall within the range of 45 to 50 mg. per kilogram which Daniels et al. ('35) suggested as being sufficient for pre-school children.

During period III wherein maximum retentions were attained by four of the subjects, approximately five-sixths of the dietary calcium was contributed by milk solids. The fluid milk equivalent approximated 440 cc. daily. Subject P apparently needed slightly more—possibly as much as 490 cc. Obviously, if these children had been receiving a diet generous in vegetables (such as is usually recommended for children of their age), it is altogether probable that the addition of a pint of milk easily would have supplied enough calcium to meet the requirements of all five of them.

The contention that a pint of milk was sufficient for the calcium needs of these pre-school girls agrees with the conclusions of Daniels et al. ('34) whose subjects retained as much calcium when a pint of milk supplemented their basal food as they did when a quart of milk was used. However, it should be pointed out that their level of calcium intake, ranging from 750 to 875, was considerably higher than that required for maximum retention by the children reported in this paper. Although the quantity of milk supplements given in the two studies was approximately the same, the basal diet used by Daniels and co-workers was considerably richer in calcium.

The present study is the first reported in which an attempt has been made to determine the minimum amount of calcium which will insure maximum retention in well-nourished

children over a comparatively long period of time. Obviously, the data may not apply necessarily to all pre-school children under all physiological and dietary conditions. Further discussion of the data will be withheld until a similar study on pre-school boys (which is now in progress) is finished.

Grateful acknowledgment is made to Dr. J. B. Gillespie of the Carle Hospital Clinic who examined the subjects from time to time and to Miss Helen King and Miss Frances Utterback who were responsible for the preparation of the children's food.

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THE UTILIZATION OF CARBOHYDRATE IN HUMAN UNDERNUTRITION [1,2]

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It has been shown repeatedly that the response of a normal animal to a test meal of glucose is profoundly altered by short periods of fasting or by a diet low in carbohydrate. This has been evident from studies of the blood sugar, oxidation experiments and, in a few instances, from analyses of tissues for their glycogen content after such test meals. Since an excellent review of the literature has recently been published by Chambers ('38) only those papers which have a significant bearing on the present studies will be discussed here.

In earlier work with the respiration chamber which has been described by Newburgh et al. ('37) it was observed that individuals who had been somewhat underfed in the preliminary periods often failed to oxidize all the carbohydrate of the diet in the experimental periods in the chamber even though they were still undernourished ('37). An attempt was therefore made to determine the conditions under which the normal human subject would exhibit diminished oxidation of carbohydrate, even though the diet was significantly below the caloric requirement.

[1] The expense of this study was defrayed in part by a grant from the Horace H. and Mary A. Rackham Endowment Fund.

[2] A preliminary report of this work was presented at the meeting of the Society of Biological Chemists at Memphis, Tenn., April, 1937.

METHOD OF STUDY

The respiration chamber for human subjects was used and the gas analyses were carried out by the method of Carpenter ('33). Frequent analyses of out-door air were made and the chamber checked at intervals by means of alcohol checks. Table 1 gives the results for this chamber for the period of these experiments. The standard methods of indirect calorimetry were employed for the calculation of the heat production and the carbohydrate oxidized using the Lusk ('28) modification of the Zuntz-Shumburg table. The calories derived from carbohydrate were divided by 3.74, the caloric value of glucose, to determine the grams of carbohydrate

TABLE 1

Alcohol checks

DATE	ALCOHOL BURNED	R.Q.	CARBON DIOXIDE			OXYGEN		
			Theoretical	Determined	Recovery	Theoretical	Determined	Recovery
	gm.		*liters*	*liters*	%	*liters*	*liters*	%
10/13/36	388.1	0.664	377.5	377.4	100.0	566.2	568.1	100.3
11/19/36	354.3	0.667	344.5	342.3	99.3	516.9	512.9	99.3
2/ 9/36	357.9	0.667	348.0	346.1	99.4	522.0	519.0	99.4
Averages		0.666			99.6			99.7

oxidized. Two normal male subjects were used who lived in a special room in the laboratory. Both were students in the department of physical education and unusually intelligent and cooperative. In earlier studies made upon R.S., who was used for the greater number of the experiments, it was determined that he maintained his weight on a diet containing 3400 calories daily. The other subject, Van H., who was somewhat larger and of approximately the same age, undoubtedly needed a somewhat higher diet. All the diets used were the simple type employed in this laboratory consisting of bread, milk, butter, eggs, 40% cream, cane sugar and jelly, and were prepared in the laboratory. The preliminary feeding periods consisted of 2 or 3 days preceding the experimental day or days in the respiration chamber.

EXPERIMENTAL RESULTS

Four experiments were carried out on R.S. (nos. 1, 2, 3, 4, table 2) in which an attempt was made to deplete the glycogen stores by feeding a diet low in calories and containing varying amounts of carbohydrate. In the subsequent 24-hour period of observation in the chamber in each of these experiments, the carbohydrate content of the diet was approximately the same as in the preliminary period but the calories were reduced somewhat to keep them significantly below the heat production. The amount of carbohydrate fed varied from 52 to 133 gm. In none of these experiments, even though the caloric deficit was sometimes approximately 900 calories in each of the 2 preliminary days of feeding (exp. no. 1) and the carbohydrate as low as 52 gm. (exp. no. 2), could we demonstrate any impairment in the ability to oxidize the ingested carbohydrate.

In the next series of experiments (nos. 5, 6, 7, 8) the subject was placed on a preliminary diet of maintenance calories and containing a liberal supply of carbohydrate, 265 gm. After 3 days of this diet he entered the respiration chamber for the experimental period. In all of the experiments of this group, the first day of observation in the chamber the subject fasted, in order that we might measure the amount of the depletion of carbohydrate. The details of this group of experiments, in which the subject remained in the chamber 3 to 5 days may be found in table 2. It will be seen that in spite of the attempt to start the period of fasting with a sufficient and, we had hoped, an equal glycogen reserve, the carbohydrate oxidation on the day of fast varied from 84 (exp. 5) to 152 (exp. 8) gm. and did not vary directly with the heat production. This must be due to variations in the activity of the subject in the preliminary feeding periods so that we did not start each experiment with the same glycogen reserve. In experiment 8 we attempted to increase the stores by overfeeding in the prelimniary period and it will be observed that although the depletion on the fast day is the largest, no storage resulted on subsequent feeding. This is likewise true of

TABLE 2

SUBJECT AND EXP. NO.	DATE	PREPARATORY PERIOD		IN CHAMBER—24 HOURLY PERIODS						CARBOHYDRATE		
		Diet	Carbohydrate	Diet	Heat production	Caloric balance	Urinary N	Total CO_2	Total O_2	Ingested	Oxidized	Balance
		cal.	*gm.*	*cal.*	*cal.*	*cal.*	*gm.*	*liters*	*liters*	*gm.*	*gm.*	*gm.*
	1937											
R.S.	Feb. 18–19	1523	265									
1	20			1523	1875	— 352	13.4	317.3	396.1	133	137	— 4
	21			1523	1825	— 302	11.7	314.9	382.9	133	173	— 40
R.S.	Mar. 29–31	1893	52									
2	Apr. 1			1755	1953	— 198	12.4	318.3	415.5	52	82	— 30
R.S.	Mar. 14–15	2526	107									
3	16			1518	1985	— 467	11.9	326.6	421.1	97	103	— 6
R.S.	Mar. 20–22	2526	107									
4	23			1720	2096	— 376	12.0	347.7	443.1	98	132	— 32
	Feb. 8–10	3500	265									
R.S.	11			Fast	1898	—1898	8.9	308.0	402.8	0	84	— 84
5	12			1378	1843	— 465	13.2	309.1	390.3	97	119	— 22
	13			1518	2011	— 493	11.2	332.5	424.6	133	120	+ 13
	1936											
	Nov. 27–28	3500	265									
	29			Fast	1935	—1935	9.5	324.8	407.3	0	146	—146
R.S.	30			1518	2236	— 718	13.5	357.0	477.8	97	53	+ 44
6	Dec. 1			1518	2073	— 555	11.5	343.4	438.6	97	124	— 27
	2			1518	2174	— 656	9.7	350.4	461.8	97	86	+ 11
	3			1518	2250	— 732	13.2	362.0	479.8	97	70	+ 27

	1937											
	Jan. 29–30	3500	265									
R.S.	31			Fast	1979	—1979	11.9	323.6	420.5	0	90	— 90
7	Feb. 1			2139	2123	+ 16	11.7	347.3	450.4	160	104	+ 56
	2			2139	2025	+ 114	9.5	338.2	427.0	160	144	+ 16
	3			2139	2038	+ 101	9.1	345.6	427.5	160	177	— 17
	Feb. 25–26	3905	290									
R.S.	27			Fast	1706	—1706	8.0	290.1	357.7	0	152	—152
8	28			1518	1970	— 452	11.8	323.1	418.4	97	95	+ 2
	1936											
	Dec. 9–10	3500	265									
	11			Fast	1942	—1942	8.1	325.2	408.4	0	146	—146
R.S.	12			Fast	1906	—1906	11.7	307.0	406.5	0	60	— 60
9	13			2139	2090	+ 51	11.8	348.5	441.5	160	137	+ 23
	14			2139	2176	+ 37	8.7	356.8	462.6	160	126	+ 34
	15			2139	2144	+ 5	8.0	351.4	451.8	160	123	+ 37
	Oct. 24–25	1512	49									
R.S.	26			1512	2102	— 590	11.2	325.6	451.4	49	0	+ 49
10	27			1512	2066	— 554	10.2	325.4	441.8	49	33	+ 16
	1937											
R.S.	June 8	0	0									
11	9–10	1755	52	1728	1911	— 183	12.9	314.0	406.3	123	90	+ 33
	Apr. 1	0	0									
	2–3	1893	52									
Van H.	4			1893	2186	— 293	16.8	352.6	468.0	87	56	+ 31
12	5	0	0									
	6			1882	2143	— 261	16.2	344.0	459.0	123	49	+ 74

experiment 5, while in 6 and 7 significant storage of glycogen resulted in the feeding periods subsequent to the fast and the amount of carbohydrate which failed to be oxidized is not directly related to either the calories ingested in the period after the fast or to the carbohydrate. In one case 82 gm. and in the other 72 gm. failed to be oxidized. In view of the limitations of the method one cannot attribute any significance to such a difference. In experiment 6 the caloric deficit in the feeding period averaged 702 calories and the carbohydrate ingested was 97 gm. while in experiment 7 the subject was slightly over-fed and ingested 160 gm. of carbohydrate.

One experiment, no. 9, was conducted in which the same preparation as above was used but was then followed by 2 days of fasting in the chamber and this in turn succeeded by 3 days of feeding with a diet containing 160 gm. of carbohydrate and exactly maintenance calories. Here 200 gm. of carbohydrate were oxidized in the 2 days of fast and storage of carbohydrate of approximately equal magnitude each day, accompanied resumption of feeding.

The demand of the organism for glycogen or the impairment of the ability to oxidize carbohydrate appeared therefore to be related to the degree of depletion of the previously existing stores. To accomplish sufficient depletion another program was tried—1 day of fast while the subject carried out his usual activity outside the chamber. This necessitated the expenditure of approximately 3400 calories and was therefore more drastic than the fast day in the chamber in which the maximal heat production was 1979 calories. This single day of fast was followed by 2 days of a diet containing 1512 calories and 49 gm. of carbohydrate (exp. 10). The 2 subsequent days were spent in the chamber and on both days the subject failed to oxidize the carbohydrate of his diet, oxidizing little or none the first day although his caloric deficit was 590 calories, and failing to oxidize but 16 gm. the second day. Exactly the same procedure was followed in experiment 11 except that the undernutrition was not quite so severe and only one 24-hour observation was made in the chamber. The

diet ingested in the chamber contained 123 gm. of carbohydrate. The storage of carbohydrate in this case was only 33 gm., no greater than in the last experiment even though the carbohydrate available was greater and the caloric deficit smaller.

In the attempt to emphasize this relationship between the previous degree of depletion of the carbohydrate reserves and the failure to oxidize a given amount of ingested carbohydrate when the caloric deficit was decreased somewhat by confinement in the respiration chamber, a second subject, Van H., was subjected to a similar but somewhat more drastic regime. In experiment 12 he fasted for 1 day during which he carried out his usual program of activity outside the chamber. This was followed by 2 days on a diet containing 52 gm. of carbohydrate and 1893 calories. On the fourth day he was given 1893 calories containing 87 gm. of carbohydrate and observed in the respiration chamber. He failed to oxidize 31 gm. of the 87 ingested. The fifth day he again fasted outside the chamber and on the sixth day entered the chamber again where he received 1882 calories containing 123 gm. of carbohydrate. This time he failed to oxidize 74 gm. of the ingested carbohydrate. A possible error exists in the calculation of the carbohydrate oxidized in this last experiment. The nitrogen of the diet on the last experimental day was 13.8 gm. The subject excreted 16.2 gm. of urinary nitrogen for this 24-hour period. Some of the nitrogen may have represented delayed excretion of nitrogen resulting from the protein metabolism of the previous fast day. If, however, one calculates the carbohydrate oxidized using the nitrogen of the diet rather than the urinary nitrogen for this period, one still finds a failure of oxidation of 55 gm. The true value may lie between the two but the interpretation is not affected by such a difference.

DISCUSSION

In these experiments the failure to oxidize all the ingested carbohydrate might be due to 1) an impairment of the oxidative mechanism, 2) an increase in the rate of storage of glycogen over the normal, or 3) a failure to convert glucose to

glycogen at a normal rate with subsequent failure of oxidation if glycogen synthesis is an obligatory step in the metabolism of carbohydrate. Chambers ('38) has summarized the literature and there seems to be little evidence that the organism in the early stages of carbohydrate depletion has any difficulty storing glycogen either in the muscles or liver. It should be pointed out, however, that most of the experiments deal with a glucose test meal, while in these experiments no carbohydrate was fed in this simple form. The carbohydrate of the diets used consisted of starch, lactose and sucrose. With regard to the first two possibilities, viz., decreased ability to oxidize carbohydrate and increased demand for glycogen by the tissues which have been previously depleted, it seems probable that both mechanisms are important. Cori and Cori ('28), in most carefully conducted experiments in which complete carbohydrate balances were made, found that rats which had been fasted 48 hours oxidized relatively less glucose and deposited relatively more glycogen than did those fasted 24 hours. This difference in deposition of glycogen was chiefly in the muscles. It may be that the impairment of the ability to oxidize glucose is associated with loss of glycogen from the muscles. In order to demonstrate failure of oxidation of carbohydrate or increased storage in these experiments, it was necessary to subject the individuals to a single fast day followed by a diet low in carbohydrate, or to 2 days of fast. Moderate depletion of the carbohydrate stores was not sufficient. The decreased ability to oxidize carbohydrate persisted for 24 hours or longer in some experiments, even though the carbohydrate was ingested in six equal meals at 4-hour intervals throughout the 24 hours, and even though the calories ingested were far below the energy requirement. The retention of carbohydrate under these conditions suggests the necessity for an optimal level of carbohydrate reserves for normal oxidation.

The interpretation of experiments of this type depend upon the validity of the method employed. The standard methods of calculation of indirect calorimetry were used for the calculation of the carbohydrate oxidized. It must be remembered

that the respiratory quotient is a resultant of metabolic processes which may include interconversions of various foodstuffs as well as oxidative mechanisms. The method employed assumes it to be a pure combustion ratio or at least to such a large extent that other reactions influence it but little. If this be true, the calculation of carbohydrate oxidized is substantially correct. Adams and Poulton ('37) have shown by an analysis of experiments in which both direct heat has been measured in the calorimeter and heat production calculated from the respiratory exchange for simultaneous periods, that a tendency exists for the indirect heat to be higher at low respiratory quotients and lower at high quotients than the direct heat. Good agreement between the two methods occurs at respiratory quotients around 0.785. They believe that carbon dioxide bears a more direct relationship to heat production than does oxygen and postulate that carbohydrate and fat are burned at a fixed ratio in the body and therefore yield a fixed combustion ratio. Values below this mean conversion of fat to carbohydrate and above that the reaction carbohydrate to fat predominates. The evidence for such a theory is not convincing. However, in these experiments the respiratory quotient ranges close to that at which the calculated heat equals the direct heat measured in the calorimeter experiments and no large error should be present in calculating the heat production in the standard manner. No quotients below that of fat were noted.

Presumably here we are not concerned with the conversion of carbohydrate to fat since in the majority of experiments submaintenance diets were fed, and in the remainder, maintenance. If conversion of fat to carbohydrate occurs with the subsequent oxidation of the carbohydrate no effect on the respiratory quotient would be seen. If, however, the glucose newly formed were stored as glycogen, which might be the case under these conditions of previous depletion, the observed respiratory quotient might be lower than if it were a true combustion quotient. If such a conversion occurred its influence upon the 24-hour respiratory quotient would undoubtedly be small and therefore of but slight significance.

Further, since no conclusive evidence exists that such a conversion does occur, the standard methods of calculation seem to furnish the best evidence for the materials oxidized we can, at present, obtain.

SUMMARY AND CONCLUSIONS

The oxidation of carbohydrate by normal human subjects has been studied in the respiration chamber following various degrees of depletion of the carbohydrate reserves. The failure to oxidize all the ingested carbohydrate appears to be related to the degree of depletion and will result when the depletion is severe enough even though the calories of the diet are significantly below the maintenance requirements of the subject.

Impairment of oxidation occurred when the stores were greatly reduced by sharp reduction of the intake of calories and carbohydrate. One might expect that the organism under these circumstances would oxidize all the incoming carbohydrate for energy purposes. On the contrary, it stores considerable portions, even though the energy expenditure far exceeds the intake. Apparently it is more important to replenish the carbohydrate stores than to use the incoming carbohydrate for fuel.

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A NOTE ON THE VITAMIN B_1-SPARING ACTION OF FAT

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The evidence in the literature, that fat in some way 'spares' vitamin B_1, has been adequately reviewed by Cowgill ('34, '38). Two interpretations have been placed on this finding, a) thiamin is not required in the metabolism of fat and, when this foodstuff is metabolized in large quantities, the amount of vitamin required is less; b) thiamin is actually present in the fat. Even though interpretation (a) is accepted, the value of feeding fat is comparable to administering a certain amount of the vitamin. Accordingly, Cowgill ('34) has expressed this sparing action in terms of 'equivalence of vitamin,' which in the case of lard is 0.55 international units per gram (1.4 micrograms of thiamin chloride).

The idea that vitamin B_1 may be present in fat has received much support from investigations using fat solvents to prepare thiamin concentrates from naturally occurring sources. Myers and Voegtlin ('20) were able to extract the antineuritic substance from autolyzed yeast filtrate with olive oil and oleic acid. Because this substance was found to react like an organic base Van Veen ('32), Block and Cowgill ('32) were able to devise a procedure for the isolation of the vitamin based upon the principle that as the free organic base it is capable of extraction from alkaline aqueous solutions by organic solvents. Subsequently, Cook and Carroll ('36) found

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that fatty acid salts of oleic or caproic acid with vitamin B_1 can be made and that these salts may then be extracted by the same fatty acids. In view of these reports it is reasonable to believe that the so-called 'vitamin-sparing action of fat' may be due to the actual presence of thiamin in fat. The conclusive experiment for the justification of this point of view would be to isolate the vitamin from fat. Obviously such an experiment is not feasible because of the fact that the thiamin concentration in fat, if present at all, is exceedingly small. However, a procedure, which should be of equal value, is the analysis of fat for thiamin by a sensitive and specific method applicable to the determination of the vitamin in this foodstuff. For this reason we have applied our chemical method (Melnick and Field, '39 a) to the assay of lard for its thiamin content.

EXPERIMENTAL

One hundred grams of lard,[2] to which 1 drop of concentrated sulfuric acid had been added, were melted on a steam bath. An equal volume of methyl alcohol was added and the mixture shaken vigorously in a separatory funnel. After the addition of 50 cc. of acidulated water and two ether extractions of the mixture, the aqueous phase was concentrated in vacuo until free from organic solvents, the pH adjusted to 4.5 and the diluted solution (100 cc. in volume) subjected to zeolite adsorption. No thiamin was present in the eluate. This experiment was repeated but this time 100 micrograms of thiamin chloride were added to the melted lard; 97 micrograms were recovered.

Because it is conceivable that the vitamin may exist to some extent as a free base at the pH of the body, and because as a free base it can form salts with fatty acids (Cook and Carroll, '36), tests were conducted on lard to determine whether any thiamin was present in such a form. If the vitamin were present as the fatty acid salt, simple aqueous extraction of the organic layer might have been unsatisfactory for the recovery of the thiamin. This belief is based upon our

[2] Obtained from Swift and Co., Chicago, Ill.

experience with phenol as an immiscible solvent for the extraction of the vitamin from aqueous solutions. Phenol has an extremely high solubility for vitamin B_1 (Greene and Black, '37). However, the quantitative recovery of the vitamin from the organic phase is exceedingly difficult. Thiamin was found to react with the solvent to form a phenolate which resists aqueous extraction despite the addition of large quantities of ether to the mixture; recoveries of the vitamin when such a procedure was employed varied from 20 to 50%. Only by use of a strong acid solution, such as 3.5 N H_2SO_4, in place of the aqueous phase previously used, were good recoveries possible, in the neighborhood of 90%. This principle was applied to the determination of thiamin in 100 gm. quantities of lard.

To the lard-methanol mixture, prepared as in the above tests, 4 volumes of ether were added and the solution extracted three times with 3.5 N H_2SO_4. The pooled acid extracts, totaling 100 cc. in volume, were carefully neutralized approximately at 5°C. to pH 2, concentrated in vacuo, the Na_2SO_4 precipitated with ethyl alcohol (80%) and removed by filtration, and finally the alcohol distilled in vacuo. The aqueous solution remaining was adjusted to pH 4.5, diluted to 100 cc. and subjected to zeolite adsorption (Melnick and Field, '39 a). In this case also no thiamin was found in the eluate. In order to test the adequacy of the procedures used, 100 micrograms of thiamin chloride were added to another 100-gm. sample of the lard and the test repeated. An 85% recovery of the added vitamin was obtained.

No analyses for the presence of the phosphoric esters of thiamin in lard were carried out because of the insolubility of such forms in organic solvents, even as the thiochrome (Kinnersley and Peters, '38) or diazo derivatives (Melnick and Field, '39 b) of the esters.

SUMMARY

Chemical analyses have indicated that lard contains no thiamin, justifying the conclusion that the vitamin B_1-sparing action of fat cannot be attributed to actual administration of the vitamin but to the decreased metabolic requirements for thiamin when the fat content of the diet is increased.

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CHONDROITIN SULFURIC ACID AS A GROWTH FACTOR

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In the course of attempts to assay for riboflavin using chicks fed the ration proposed by Wilgus, Norris and Heuser ('35), it became apparent that this basal diet was deficient in other factors besides riboflavin. Many lots of chicks failed to grow on the positive control ration (riboflavin [1] supplemented), symptoms typical of anti-dermatosis factor deficiency often appeared in both positive and negative controls and the 'curly toe' type of paralysis described by Norris, Heuser, Wilgus and Ringrose ('31) was seen in some of the low-level positive controls. The addition of a rice-polish filtrate decreased the death loss, abolished the dermatosis and produced some improvement in growth. However, growth did not appear to be optimal even when the basal ration was supplemented with both riboflavin and rice-polish filtrate.

Since the present authors ('38) had previously found that chondroitin sulfuric acid (hereafter referred to as CSA) had a favorable effect on growth in some instances when added to diets which produce gizzard erosion in the chick, and because the properties of CSA bear some resemblance

[1] The riboflavin used throughout these experiments was crystalline lactoflavin obtained from the Borden Company.

to those described by Jukes ('37 a) for the chick anti-dermatosis factor, it has been our purpose to determine the effect of adding CSA to the riboflavin assay basal ration.

Chondroitin sulfuric acid (CSA) is the prosthetic group of chondromucoid (a protein of cartilage), and is composed of glucuronic acid, galactosamine to which an acetyl radical is joined, and sulfate groups. Crandall, Roberts and Gibbs ('32) and Crandall, Roberts and Snorf ('36) have shown that CSA had a systemic effect as evidenced by its therapeutic activity in migraine and the improved nutritional condition it produced in Eck fistula dogs. However, there is but one report suggesting that CSA (or one of its components) is of value in correcting dietary deficiencies. Bird and Oleson ('38) have reported that CSA prevented gizzard erosion, an observation which we have failed to confirm ('38) even though a large number of animals were studied on various erosion producing diets.

EXPERIMENTAL

In all of our present experiments involving chicks, day-old Barred Rocks were placed on the basal ration (or this ration supplemented as indicated) for a depletion period of 2 weeks. At the end of this time they were weighed, wing-banded and so placed in groups that average lot weights were comparable. About 30% were discarded as too light or too heavy, only chicks of medium weight being used. Each test lot contained between fifteen and twenty chicks. They were kept in temperature-controlled screen bottom cages in an air-conditioned room and were allowed free access to food and water.

The basal ration employed was that suggested for riboflavin assay by Wilgus, Norris and Heuser ('35). It consisted of: yellow corn 54, wheat middlings 20, vitamin-free casein 16, dextrin 4, cottonseed oil 3, steamed bone meal 2 and cod liver oil 1. Jukes ('37 b) modified this diet by supplying the anti-dermatosis factor from rice by-products. The rice polish filtrate used by us was prepared by extracting the polishings for 24 hours with 25% alcohol, acidifying and

treating with fuller's earth to remove riboflavin. Completeness of riboflavin removal was checked photometrically.

In the first three groups of animals, the basal diet was given for 2 weeks, at which time supplements of rice-polish filtrate, rice-polish filtrate and riboflavin, and CSA and riboflavin were added. The results are given in table 1 and show that a much better growth and decreased death loss resulted from the CSA and riboflavin supplement as compared to the other two groups.

TABLE 1

Growth in chicks receiving supplements of rice polish filtrate and flavin as compared to CSA and flavin

TEST NO.	SUPPLEMENT	AVERAGE WEIGHT AT START (2 WEEKS)	AVERAGE WEIGHT AT 4 WEEKS	AVERAGE WEIGHT AT 6 WEEKS	GAIN	DEAD
		gm.	*gm.*	*gm.*	*gm.*	*%*
41S	Rice polish filtrate [1]	63	65	114	51	55
41T	Rice polish filtrate [1] and and 150 micrograms riboflavin per 100 gm. ration	65	104	158	93	33
41Z	3% CSA plus 150 micrograms riboflavin per 100 gm. ration	65	122	229	164	0

[1] Equivalent to 20% addition of rice polishings.

We next wished to determine whether CSA is identical with the chick anti-dermatosis factor. Dermatosis is readily produced by using a heated ration according to the method of Kline, Keenan, Elvehjem and Hart ('32). Accordingly, the corn, middlings, casein and dextrin were heated at 115°C. for 64 hours before incorporation in the basal ration. Three lots of chicks were selected after the previously outlined preliminary period of 2 weeks and were given supplements as in the first three groups. The results are given in table 2.

Dermatosis appeared as expected. The chicks receiving CSA and riboflavin showed a slightly better weight gain than those receiving riboflavin alone, but there was no alleviation of the dermatosis. Rice-polish filtrate produced the best gain

and prevented dermatosis. It is evident that CSA is not the chick anti-dermatosis factor. The poor growth in all three of these groups is possibly due to destruction of amino acids in the casein by heat.

The next experiment was designed to determine the optimum amount of CSA for the chick when added to the basal ration. For this purpose, riboflavin (250 micrograms per 100 gm.) was added to the basal ration throughout the 2-week depletion period as well as during the remainder of the test. Purified CSA was used as a supplement in amounts up to 3% of the

TABLE 2

CSA in the heated basal ration

TEST NO.	SUPPLEMENT	AVERAGE WEIGHT AT START (2 WEEKS)	AVERAGE WEIGHT AT 4 WEEKS	AVERAGE WEIGHT AT 6 WEEKS	GAIN	DEAD
		gm.	gm.	gm.	gm.	%
43J1	250 micrograms riboflavin per 100 gm. ration	53	52	52	−1 [1]	80
43J2	250 micrograms riboflavin per 100 gm. ration plus 3% CSA	51	60	78	17 [1]	66
43J3	250 micrograms riboflavin per 100 gm. ration plus rice polish filtrate [3]	51	66	99	48 [2]	62

[1] Dermatosis present.

[2] No dermatosis.

[3] Equivalent to 20% addition of rice polishings.

ration. The CSA was purified by hydrolysis of the commercial product with 2% sodium hydroxide for 24 hours, precipitation with slightly more than 1 volume of alcohol, solution in water, and reprecipitation with 6 volumes of glacial acetic acid. It was then washed thoroughly with alcohol and ether and drying completed in vacuo. The glucuronic acid content of the purified material was 36.0%; theory requires 37.6%. Growth-promoting effects of the several levels of CSA supplementation are recorded in table 3. The optimum amount appears to be about 2 to 3%; improved growth is produced by the lowest level (0.5%).

The gizzards of all the chicks used in the tests listed in table 3 were carefully examined. When erosion was found, they were graded according to the following score: 1, no lesion; 2, slight erosion; 3, marked erosion and 4, severe erosion. The average scores for the various groups were

TABLE 3

The effect of varying levels of CSA on chick growth

TEST NO.	CSA [1] ADDED	AVERAGE WEIGHT AT START (2 WEEKS)	AVERAGE WEIGHT AT 4 WEEKS	AVERAGE WEIGHT AT 6 WEEKS	GAIN	DEAD
	%	gm.	gm.	gm.	gm.	%
44F	None	78	95	110	32	13
44G	0.5	80	105	152	72	0
44H	1.0	80	120	174	94	0
44I	2.0	80	130	191	111	0
44J	3.0	79	128	197	118	0

[1] Purified CSA added to basal ration containing 250 micrograms riboflavin per 100 gm.

TABLE 4

CSA supplementation of the basal ration using white rats [1] as test animals for a 7-week growth period

TEST NO.	SUPPLEMENT	WEIGHT AT START	WEIGHT 7 WEEKS	AVERAGE GAIN	GRAMS FEED PER GRAM GAIN
		gm.	gm.	gm.	
442	None	56	145	89	5.12
443	3% CSA	59	182	123	4.22
444	3% CSA plus 150 micrograms riboflavin per 100 gm. basal	55	180	125	4.25
445	150 micrograms riboflavin per 100 gm. basal	57	169	112	4.46

[1] Two male and four female rats, 4 weeks old, and evenly distributed by litter were used for each test lot.

2.9 for the basal ration plus riboflavin but no CSA, and 3.2, 3.4, 3.6 and 3.4 for the 0.5, 1.0, 2.0 and 3.0% levels of CSA, respectively. This finding that the severity of the lesions is similar irrespective of the level of CSA fed confirms our previous observation ('38) that CSA is without effect on gizzard erosion.

To determine whether the growth-promoting effect of CSA is peculiar to the chick or whether it can be observed in mammalian forms as well, the experiments represented in table 4 were carried out with rats. The basal diet alone and supplemented by 3% CSA, 150 micrograms of riboflavin per 100 gm. of ration and 150 micrograms of riboflavin per 100 gm. of ration plus 3% CSA was fed to four groups. Each group was composed of two male and four female rats 4 weeks old.

It will be noted that when CSA was the only supplement the growth rate is much improved over that on the basal diet alone, and is as effective as the combination of CSA and riboflavin. It is questionable whether the difference between the group on riboflavin alone and those on CSA or CSA and riboflavin is significant.

DISCUSSION

It is evident from our data that CSA has a definite growth-promoting effect on the chick and the rat when added to the basal ration we have employed. Since the purified CSA gave no tests for protein or polypeptides and contained no known dietary essential as an impurity, the growth stimulation obtained must be attributed to the CSA molecule as such or to the glucuronic acid or galactosamine of which it is composed. Further experimentation is now in progress to determine whether either of these constituents is effective. It should be recalled that there is no known mechanism by which chondroitin may be split into its constituent parts. A sulfatase has been described which splits off sulfate radicals from CSA, but no enzyme has been found which is capable of hydrolyzing the remainder of the molecule into six carbon chain fragments.

We believe that CSA is not identical with any growth factors previously identified. The data show that it is not the anti-dermatosis principle and supports our previous contention ('38) that it is ineffective in gizzard erosion in the

chick. CSA is capable of promoting growth when presumably adequate amounts of other known dietary essentials were present in the basal ration.

SUMMARY

Chondroitin sulfuric acid has been found to serve as a growth factor for the chick and rat. This material is not identical with the anti-dermatosis factor found in rice-polish filtrate. A 3% level of CSA was apparently optimal for growth in the basal ration used but did not prevent the occurrence of well-defined gizzard lesions.

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EDITORIAL REVIEW

FACTORS INFLUENCING THE APPARENT ENERGETIC EFFICIENCY OF PRODUCTIVE PROCESSES IN FARM ANIMALS

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1. The influence of body size on apparent efficiency of milk production, egg production, growth and muscular work

In 1901 Jordan published in his book 'The Feeding of Animals' the following table:

TABLE 1

Efficiencies of productive transformations in farm animals (Jordan)

Animal and product	*Edible solids in pounds per 100 pounds of digestible organic matter in the ration*
Cow, milk	18.0
Hog, carcass	15.6
Calf, carcass	8.1
Fowl, egg	5.1
Fowl, carcass	4.2
Steer, carcass	2.8
Sheep, carcass	2.6

Jordan's table indicates that body size, species and nature of product are factors in the efficiency of productive processes. Popular belief supports this impression. The question of relative efficiency of milk production in large and small cows and (still smaller) goats is under lively debate. Similar discussions are popular as regards influence of size on other productive processes and breed associations advertise their favorite animals on the basis of their supposed superiorities.

Paper 179, Herman Frasch Foundation for Research in Agricultural Chemistry; paper 573, Missouri Agricultural Experiment Station, Department of Dairy Husbandry.

To the sponsor of the small animal the superiority of the (small) goat over the (large) cow is self evident. A 120-pound goat may attain the productive level of 15 pounds milk a day (1) whereas it appears altogether improbable that a 1200-pound cow could produce 150 pounds milk a day. Hence the conclusion that a small animal as a goat is superior as a milk producer to a large animal as a cow, and that perhaps the smaller the animal the higher the relative productivity. To clinch his argument, the sponsor of the small animal cites the rat, which in 3 weeks of lactation raises its newborn litter to a weight roughly equal to her own (2). As compared to the cow, or even to the goat, the milk production in the rat, in comparison to her body weight,[1] must be collosal.

The sponsor of large animals also finds plausible arguments to support his favorites. In the first place the basal metabolism per unit body weight is known to be less in large than in small animals (3), and therefore the maintenance cost per unit body weight is likely to be less in large than in small animals. If the milk production is proportional to maintenance cost, then the gross efficiency,[2] that is the amount of milk produced per unit feed consumed (rather than per unit body weight), is the same in large and in small animals; and if the gross efficiency and price per unit product are the same in large as in small animals, then the large animal is preferable because the management expenses (housing, milking, bookkeeping, etc.) are less in handling a few large, than many small, animals producing the same amount of milk in total (10).

Non-partisan considerations might perhaps lead to the expectation that there should be no difference in gross efficiency of productive processes in large and small animals. In the

[1] Note that both sides overlook one aspect of the time element. A (small) rat or goat may produce more milk energy per unit body weight per day during a lactation period than a (large) cow, but what are the relative life-time productions per unit body weight, per unit basal metabolism, per unit maintenance expense in large and small animals? What are the relative lactation-period daily maxima and life-time productivities and gross efficiencies of small and large animals?

[2] $$\text{Gross efficiency} = \frac{\text{output energy (Cal.)}}{\text{input energy (Cal.)}} \qquad (1)$$

first place, if there were such size differences, the animal of the most efficient size would likely survive in the struggle for existence at the expense of the less efficient one. In the second place, if, as seems reasonable, there are no size differences in ability to assimilate and metabolize food, and it is not costlier to organize, for example, milk from nutrients in large than small animals, then the net efficiency [3] would be the same, and the gross efficiency [2] of the milk production might also be expected to be the same in large and small animals provided however that the milk-energy production and maintenance-energy cost change at the same relative rates with increasing body weight. The crucial question thus becomes: What is the relative influence of body weight on: 1) maintenance cost, and 2) productive level?

While there is general agreement on how basal metabolism varies with body weight (3) there is no agreement on how the feed cost of maintenance varies with body weight, because of the possibly variable activity increments with changing weight in different individuals and species. However, Kleiber (4) on the basis of extrapolation of limited data concluded that maintenance, productivity and basal metabolism all increase at the same relative rate, namely with the 3/4 power of body weight, and that consequently gross energetic efficiency of transformation is independent of body weight.

The lively interest among animal husbandmen and others concerning the influence of body size on productivity and efficiency, the practical importance and theoretical interest in the problem of efficiency of biologic transformations, and the contrast in the prevailing opinions on the problem led us to investigate it on: milk production in small and large dairy cows (5) [a problem pioneered by Gaines (6)], dairy goats (1), and rats (2); egg production in large and small fowls (7); muscular work in large and small horses and men (8); growth in small and large species of animals (5, 7); wool production in small and large sheep (9); lamb production in small and large sheep. While the results of these investi-

[3] Net efficiency $= \frac{\text{output energy}}{\text{input energy less maintenance energy}}$ (2)

gations are altogether tentative, they may be of interest in this connection.

The essential results on milk and egg production are summarized in table 2, which shows that within the limits of expected biologic variability, and experimental and computational errors: 1) the ratio of milk energy produced (in naturally high-milking animals) to digestible feed energy consumed (that is, gross energetic efficiency [2] of milk production) is not only independent of live weight within the species, but even between species; 2) the ratio of milk energy produced to basal metabolism energy is independent of live weight, from which it follows that 3) the ratio of total maintenance energy cost to basal energy metabolism is independent of live weight or even of species—it is (within the aforenoted limits of variability) the same in cows, rats and goats. We may thus conclude this discussion on milk-production efficiency as influenced by body size with the very important tentative generalization that maintenance-energy basal-metabolism energy and milk-production energy all tend to increase at the same relative rate with increasing body weight, and the gross energetic efficiency of milk production tends to be independent of body weight as such or even of species as such.

As previously noted and as shown in table 2, the intensity of milk production measured by the ratio of milk energy to body weight is very much greater in small than large animals (the ratio milk-calories produced per day to body weight in kilogram is of the order of 200 in rats and only 25 in dairy cows), but there are similar relative differences in the intensity of metabolic and consequently probably in maintenance energy expenditure, so that changing maintenance energy and milk energy with increasing body size just compensate, with the net result that the gross efficiency is the same in large and small animals despite the fact that per unit body weight milk-energy production is very much greater in small than large animals.[4]

[4] It should be noted, however, that while energetic efficiency (a ratio) may not depend on body weight, monetary profit (a difference) is likely to. If gross efficiency and price of milk are the same, the larger the dairy cow, for example, the smaller the overhead cost of management per unit product and consequently the greater the profit (10).

TABLE 2

Gross energetic efficiency (with respect to total digestible nutrients consumed) of milk production in dairy cows, white rats and dairy goats (and related data); also egg production in fowls

	MILK PRODUCTION						EGG PRODUCTION	
	Dairy cows (5, 10)			White rats by methods A and B (2)		Goats (1)	Chickens (7)	
	'Ordinary' (superior) all breeds	'Extraordinary'		Arithmetic average of all litters				
		Champion Holstein	Champion Jersey	A	B		Ordinary fowls	Champion fowls
Number of cows, rat litters, goats, chickens	368	1	1	7	5	7	478	1
Body weight, kg.	513	771	318	0.291	0.295	42.5	1.7	1.8
Average milk (or egg) production Cal./day (length lactation period ranged in cows from ½ to 1 year; in rats between 10 to 18 days lactation inclusive on 4- to 9-rat litters; goats from 11 to 25 weeks)	11,440	34,000	24,140	71.5	60.1	2114	49.4 Cal. egg/day	90 Cal. egg
Gross energetic efficiency of egg or milk production with respect to TDN energy, %	31	44	48	48	44	34.9 (32.0–40.5)	16	27
Estimated basal metabolism, Cal./day	6,700	9,000	4,700	29	29	1100	104	108
Ratio basal metabolism Cal./day to body weight, kg.	13	11.7	12.3	99	99	26	61	60
Ratio milk or egg Cal. to basal metabolism Cal.	1.7	3.8	5.1	2.1	2.1	1.9	0.48	0.83
Ratio milk or egg Cal. to body weight, kg.	22	44	63	207	149	49.8	29	50
$(Wt.)^{0.73}$	95.1	128	67.1	0.406	0.411	15.4	1.473	1.536
Ratio milk or egg Cal. to $(Wt. Kg.)^{0.73}$	120	266	360	148	147	136.9	33.5	58.6

A comparison of the productivities and efficiencies of egg (7) and milk (5) production in the right column of table 2 shows that for roughly comparable producers, the gross energetic efficiency [2] (16%) of egg production is about one-half that for milk production (31 to 35%). However, the net efficiency [3] (not counting maintenance cost) is of the same order for egg and milk production (the net efficiency with respect to TDN [5] energy consumption is from 60% to 70% for both milk and egg production (5, 7) while the top gross efficiency is shown in table 2 to be of the order of 27% for eggs and 48% for milk production).

The fact that net efficiency is probably the same in milk and egg production means that the mammary gland produces milk from TDN[5] with the same efficiency as the ovary-oviduct system produces egg. The fact that the gross efficiency [2] is less for egg than for milk production means that in comparison to maintenance expense less egg energy is produced in fowls than milk energy in cows. The possible explanation for the lower egg-energy production in comparison to maintenance cost than of milk-energy production, may be due to: 1) greater structural complexity in egg than milk (7); 2) as a result of 1) more biologically equivalent time (11) is taken to

[5] In this review the output energy is assumed to be the heat of combustion of the product, as milk, egg, meat; the input energy is assumed to be the metabolizable energy in the apparent total digestible nutrients consumed. 'Total digestible nutrients' is abbreviated in the literature to TDN, which equals to digestible carbohydrates + digestible protein + digestible fat × 2.25. It is also assumed that the thermal equivalent of the metabolizable energy is 4 Cal. per gram TDN or 1800 Cal. per pound TDN. The thermal values as reported in the literature range from about 1600 Cal. to about 1800 Cal. per pound TDN or from about 3.6 to 4.0 Cal. per gram TDN. The interested reader is referred for details to L. A. Maynard's 'Animal Nutrition,' McGraw-Hill, 1937; Armsby's 'Nutrition of Farm Animals' ('17), p. 650; Forbes and Kriss, Proc. Soc. Animal Production, 1931, p. 113; Christensen and Hopper, J. Agr. Res., Oct. 1, 1938. The efficiency computed on the assumption that 1 gm. TDN = 4 Cal. when multiplied by

becomes efficiency on basis that 1 gm. TDN = 3.6 Cal., and so on for other reference base values. It is thus easy to change efficiency from the basis of one to another thermal equivalent of TDN.

[2, 3, 5] See footnotes 2, 3 and 5 previously cited.

produce unit egg energy than unit milk energy, with consequently greater expenditure of overhead maintenance cost per calorie of egg than milk.

As regards growth, the problem of its gross efficiency [2] is complicated by the age factor. Thus according to Tangl, Glaser, Farkas, Bohr and Fauriet-Fremiet, among others, the gross efficiency of embryonic growth is of the order of 60 to 80% (12). According to Rubner and Lusk (13) the gross efficiency of early postnatal growth is (except in man) of the order of 34%. A part of this difference in result may be due to differences in muscular exercise, environmental temperature and nature and form of dietary energy. But most of the difference is due to increasing body weight and decreasing growth rate. The older the animal and the greater its size, the greater the maintenance expense of the body in comparison to weight gain. The over-all or gross energetic efficiency of a living converter is thus dependent on two factors: 1) maintenance cost which in turn depends on the size of the animal; and 2) speed of the converting process which depends on physiologic age (11) and may be dependent on the structural complexity of the product. Differences in gross efficiency of biologic transformations in productive processes—as egg or milk production, or growth—can usually be traced to these determining factors. Thus egg production is less efficient energetically than early growth, because whereas egg production includes the overhead maintenance cost of the fowl that produces the egg, growth of the chick does not include the overhead maintenance cost of its mother. With increasing age, however, a maintenance charge is built up by the increasing size of its body with a consequently decreased gross efficiency. Table 3 exemplifies the influence of age on estimated growth efficiency of small and large species of animals.

Table 3 shows that the over-all or gross efficiency of early postnatal growth at equivalent physiologic ages (11) is of the same order in large and small animals, and of the same order as of milk production, and higher than of egg production.

There is a decrease in gross growth efficiency with increasing age because in comparison to maintenance energy there is decreasing growth. The net efficiency of growth may not be affected by age, but gross efficiency must be.

Gross energetic efficiency of muscular exercise refers to the ratio of work performed to total energy expended as measured by oxygen consumption, rather than to the TDN-energy [5] consumption. That is, the work efficiencies do not include the cost of converting TDN to body fuel. Because of the difference in reference-base values, and in the nature of productive proc-

TABLE 3

Estimated gross efficiency of growth with respect to total digestible nutrients, TDN, consumed (5, 7)

POSTNATAL AGE (MONTHS FOR CATTLE, WEEKS FOR CHICKENS AND RATS)	GROSS EFFICIENCY, PER CENT						
	Chickens (Several groups under different dietary conditions)			Rats		Dairy cows	
	W. Leghorns	R. I. Reds	W. Leghorns	Males	Females	Holstein	Jersey
1	26	21	35	25	23	35	37
2	22	17	38	23	19	26	25
3	19	25	33	21	17	33	30
4	21	17	32	20	15	23	26
5	18	22	29	19	14	18	20
6	20	19	23	18	13	17	20
7	19	19	18			13	13
8	16	18	26			13	13

esses, the gross-efficiency values of muscular exercise are not directly comparable to gross-efficiency values for milk, or egg, or meat production. However, the maximum gross energetic efficiency of muscular work, which is near 25%, is the same in small and large horses and apparently also in man (8). So again we conclude that maintenance energy and production energy increase at the same relative rates with increasing body weight.

It thus seems permissible to conclude tentatively, that when other conditions (e.g., inheritance for maximum milk production, body fatness, food supply) are of the same order, the gross energetic efficiencies of milk production, egg production and muscular work tend to be independent of body

weight and species as such. The gross energetic efficiency of growth is probably likewise independent of body weight and species as such but only for equivalent physiological ages (11).

2. The influence of endogenous and exogenous factors on apparent gross efficiency

Variation in productivities within a given species and weight class and consequently variations in gross efficiencies may be due to two types of influencing factors of equal interest to students of gross efficiency: 1) external or exogenous, as dietary, which are the major concern of students of nutrition and biochemistry, and 2) internal or endogenous, as circulatory, respiratory, excretory, which are the major concern of students of physiology. The catalysts (vitamins, hormones, enzymes) are of equal concern to students of nutrition, physiology and biochemistry, because: a) they affect all bodily transformations (compare influence of thyroxin or insulin on metabolic—nutritive—transformations); b) functionally they all serve as catalysts; c) depending on evolutionary circumstances the same substance may be vitamin (exogenous factor); hormone, or enzyme (endogenous factor). It is thus generally known that ascorbic acid is in some species (guinea pig) obtained from food, and is therefore a vitamin; in other species (rat) it may be produced in the body possibly as a hormone,[6] and it is in all cases an oxidation-reduction enzyme in the body. Similar relations exist between most other vitamins (14), enzymes, hormones, organizers (15), carcinogens (16), depending on the conditions of the field structure. There seems to be no functional or structural dividing line between at least some of the dietary and some of the hormonal factors.

Let us then discuss the problem of variability in gross efficiency as influenced by hormonic and dietary factors, illustrating it by the milk-production process. It is generally known that milk-production levels and consequently gross-efficiency levels are very much greater in dairy than beef cattle of the same body weight, or in dairy than mohair goats.

[6] Ascorbic acid may possibly be produced in the digestive tract.

Body weight is obviously here eliminated as an influencing factor.

Broadly viewed, milk secretion is one of the many interdependent delicately synchronized mechanisms in the service of a single function, that of bridging the perilous gap between the dependent intra-uterine life and independent adult life. It has been definitely established (17) that the anterior lobe of the pituitary gland is the chief endogenous (hormonal) regulator of milk production. This gland is also the regulator of many other processes. Some investigators (17) maintain that milk secretion is the resultant of the action of a series of distinct hormones: 1) pituitary lactation hormone which regulates milk production by direct influence on the secreting cells of the mammary gland, and 2) metabolic hormones for protein, carbohydrate, fat, etc., each of which regulates milk production by its influence on a particular milk precursor. Other investigators maintain that the same biochemical entity (hormone) which stimulates milk secretion by mammary cells shows other regulating (hormonal) properties among which were reported (18) to be: 1) calorigenic in conjunction with the pituitary thyrotropic hormone; 2) calorigenic in thyroidectomized animals; 3) diabetogenic;[7] 4) crop-secretion stimulant in pigeons; 5) releaser of brooding instinct in fowls and maternal behavior in rats;[8] 6) sustainer of liver, pancreas, and intestines in pigeons; 7) participator in acceleration of growth. Indeed the lactation hormone is customarily assayed by its influence on crop growth (18) in pigeons of either sex rather than by its milk-stimulating property. The fact that the same substance which apparently stimulates mammary-gland cells to produce milk also induces maternal behavior gives a sort of philosophic functional unity to a diversity of phenomena, although similar philosophic unity could be deduced from the synchronized action of many distinct hormones.

[7] However, Bergman and Turner reported separation of lactogenic and carbohydrate-metabolism anterior-pituitary hormones (Endocrinology, vol. 22, p. 619, 1938).

[8] However, Leblond and Nelson reported maternal behavior in hypophysectomized animals (Am. J. Physiol., vol. 20, p. 167, 1937).

There are similar perplexities as regards growth hormones. There may be a specific growth hormone as postulated by Evans (19), but Riddle (18, '37) reported support for the view that bodily growth is favored by various pituitary hormones. It seems reasonable that there may be many interlocking hormones acting as a harmonious unit in stimulating the process of growth and development.

Whichever the situation, it is undoubtedly possible to accelerate productive processes (lactation, growth, and so on) by hormone administration; and since increased level of productivity is normally associated with increased gross efficiency, one might perhaps hope to increase the gross efficiency of productive processes by hormone administration; or possibly by breeding animals for desirably high endocrine activity on the basis of knowledge of their hormonal constitution (17). Several questions suggest themselves in this connection: 1) Is immediate efficiency, as well as productive level, increased by hormone administration? 2) Can long-range efficiency (as contrasted to immediate efficiency) be increased by hormone administration? 3) Can long-range efficiency be increased by breeding for a special production-accelerating endocrine? Or would progress be more likely to follow selection for the desired long-time function which necessarily includes the participation of the entire organism, in the entire organic space-time field, including the aging process?

The organismic viewpoint leads to the inference that in the course of evolution the component parts of the body developed to function in organismic harmony, which would be disturbed with unfavorable after-effects, and therefore reduced long-range efficiency, either by dosing the animal with, or breeding for, one productive stimulator and leaving the remainder of the body unimproved. However, the influence of the time factor is not predictable. Thus the unfavorable after effects may not become marked until after the animal has reached the end of the desired productive period. This complex problem of immediate versus long-range efficiency needs care-

ful formulation and investigation. Let us illustrate the possible interrelations between hormone administration and productive efficiency by the influence of thyroxin injection on lactation.

The increased milk production by thyroid feeding or thyroxin injection has been attributed in part to increased circulation rate (20). Thyroxin may also increase the speed of digestive and assimilative processes (21), which are so important for production. But in addition to speeding up circulation, digestion and assimilation, thyroxin administration speeds up the metabolism (maintenance item in equation 2)[9] of all systems. Thyroxin is a general metabolic accelerator that speeds the general tempo of life, of which milk production is only one manifestation; and it may be inferred from the results by M. Rubner, J. Loeb and R. Pearl (that acceleration of the rate of living accelerates the rate of senescence) that speeding up metabolism (the 'rate of living') by thyroid would accelerate senescence. Then, too, the thyroxin may conceivably overstimulate the vital organs, especially the circulatory system, thereby shortening the animal's life, as illustrated for example, by fatalities from auricular fibrillation in hyperthyroid individuals. Thus, even though thyroid administration increases the production level and might increase the immediate gross efficiency of milk production, it is not unlikely that it might also decrease the long-range gross efficiency by destroying the animal's usefulness prematurely.

Investigators of gross efficiency of transformation of energy-matter cannot overlook possible factors affecting longevity and fertility, because they enter into the reckoning of long-range efficiency. From the long-range viewpoint, it seems very important to pose such questions as: 1) Is extraordinarily rapid growth, or high productive level of any kind, brought about by dietary or/and hormonal methods compatible with maximum longevity and fertility? 2) What is the relation between successive increments of productive stimuli

[9] See footnote 3. Equation (2) in slightly expanded form:

$$\text{Gross efficiency} = \frac{\text{milk energy}}{\text{milk energy} + \text{maintenance energy} + \text{other expenses}}$$

(e.g., successively larger administrations of dietary and hormonal factors) on longevity, fertility and long-range gross efficiency as contrasted to immediate productivity and efficiency? 3) May not the price paid for immediate success in achieving relatively high levels of productivity and gross efficiency be offset by unfavorable after effects with possible long-range failure?

We chose to discuss the influence of thyroxin on productivity and gross efficiency of lactation, a typical productive transformation, not only because the properties and effects of thyroxin are best known, but also because thyroxin may be administered in chemically pure form. It would be premature to discuss the influence on long-range gross efficiency of administration of other specific production-accelerating hormones, as the lactation hormone, because they have not yet been isolated. All that we know about the lactation hormone is that injection of anterior pituitary preparation exhibits, among other physiologic effects previously noted, initiation and maintenance and, in the absence of other limiting factors, possibly increase in milk production (17).

What has been said about hormone factors affecting milk-production efficiency appears to be applicable to growth efficiency; and what has been said about hormone factors may be said about dietary factors affecting productive processes. Growth has been enormously accelerated by injecting suspensions of whole anterior pituitary or its crude alkaline extracts (19). Growth has been likewise accelerated by dietary means (22). Indeed, during the duration of the experiment, the growth rate of the dietary-accelerated rats of Anderson and Smith or even of Mendel and Cannon (20) was higher than the growth rate of the hormone-accelerated rats of Evans (19). Unfortunately, Anderson and Smith did not have data on control rats of the contemporary stock fed an 'ordinary diet' at the same time, so that it is possible that the unusually high growth rate was not altogether the result of the unique dietary factors as such or of the appetizing nature of the diet which caused them to consume more feed.

Employing the paired-feeding method, some observers recorded accelerated growth as result of hormone administration (23, p. 399), and of vitamin, protein and mineral enrichment (23, pp. 400–404) on the same energy intake as normally growing rats. These results point to greater gross efficiency of growth in the accelerated than in normally growing rats; but in the absence of complete energy-balance data, or at least analyses of the composition of the gains, it may be argued that the superior growth rates under the influence of hormone administration or of special dietary supplements are confined to water, minerals and nitrogen rather than to total energy storage (23, pp. 400–402). There is need for investigating the influence of accelerated growth and other productivities as results of special dietary and hormonic factors not only on immediate efficiency but also on long-range efficiency which must be compatible with longevity, 'highest livability,' and fertility (23, pp. 383–392).

3. The principle of optimal and limiting factors in efficiency

'Normal' growth rate associated with 'normal' body composition, longevity and fertility is a resultant of interrelationships between innumerable intrinsic and extrinsic, endogenous and exogenous, factors; between 1) organizers, hormones, enzymes, responsiveness of the component tissues, and 2) food supply and other environmental factors. Genetic and non-genetic factors cooperate in the production of every trait. Thus (24) MacDowell observed striking differences in 'genetically-pure' litter-mate mice, living in the same cage on the same diet, and Newman, Freeman and Holzinger reported striking mental and physical differences in 'identical' (monovular) human twins living under different social-economic conditions.

A necessarily common characteristic of highly efficient animals whether the production be to meat, milk or eggs, is that the animals are high consumers of feed in comparison to maintenance. But what governs high feed consumption?

We have discussed the hormonal factors involved in stimulating production rate and consequently feed consumption.

As regards the nature of the diet, it is generally known that feed consumption is decreased and feed utilization is depressed when the diet is unbalanced with respect to certain constituents (23, p. 403).

Another factor affecting feed intake and gross growth efficiency is environmental temperature (25). This factor may be used to illustrate in a simple manner the principle of optimal and limiting factors. When temperature is too low gross efficiency is reduced because too great a portion of the feed is used to keep the animal warm. When temperature is too high, gross efficiency is reduced because at the upper belt of thermal neutrality the metabolic level is very low and the productive rate is correspondingly low. Between these two limits there is a point where the feed intake above maintenance, in comparison to maintenance, is a maximum and productive rate and gross efficiency are maximal. Similar reasoning is applicable when any endocrine activity, or muscular activity, or any other factor or function is too high or too low. It may be possible to represent the interrelations between the various components in the efficiency complex in some such manner as Henderson (26) represented interrelations between various components in the blood complex. The problem, in brief, is to map the limiting and optimal interrelationships in the time-space configuration which is the productive converter of energy-matter under investigation in order to furnish the background for intelligent thinking on nutritive processes in productive transformations (27).

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THE CALCIUM REQUIREMENT OF ADULT MAN AND THE UTILIZATION OF THE CALCIUM IN MILK AND IN CALCIUM GLUCONATE [1]

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The calcium requirement of the adult organism relates to the replacement of endogenous losses of calcium from the body, mainly through the kidneys and the alimentary tract. While the cause of these losses is quite obscure, their existence has been definitely established in many published experiments on human subjects. We may consider the endogenous loss of calcium per day as measuring the requirement for maintenance. It is undoubtedly dependent upon body size, but whether it varies directly with body weight, body surface or with some other power function of body weight is not at present known. However, until this question is settled the endogenous losses of calcium may be tentatively expressed to the kilogram of body weight.

The efficiency of dietary calcium in replacing endogenous losses may be expected to vary with the kinds of food materials contained in the diet. Hence the dietary requirement of calcium for maintenance will vary with different diets and will probably always be much greater in magnitude than the endogenous loss. In a compilation and analysis by Mitchell and Curzon ('39) of available information on the subject including 139 average observations on 107 human subjects,

[1] This investigation was aided materially by the donation of funds from the Graduate School of the University of Illinois, for which grateful acknowledgment is made.

only eighteen of whom were males, it was found that the average endogenous loss of calcium was 3.1 mg. per day per kilogram of body weight, while the average intake of dietary calcium required for calcium equilibrium was 9.75 mg. per day per kilogram of body weight. Thus, slightly more than 3 gm. of dietary calcium is required on the average to replace an endogenous loss of 1 gm. But considerable disparity exists among the individual data here summarized, partly the result of metabolic differences among the various subjects studied, partly the result of the short experimental periods usually employed in human metabolism studies, and partly the result of the many different types of diets employed, containing different sources of calcium.

It seems important to know the extent to which the calcium in human foods is utilized by the adult, particularly those foods that supply important amounts of calcium to the American diet. Most of the experimental evidence concerning the utilization of the calcium of foods by human subjects reviewed by Mitchell and Curzon ('38) is of comparative, not absolute, significance. In none of the experiments on adults there reviewed is it possible to compute from the reported data the per cent to which the dietary calcium was used in the body in the replacement of endogenous losses of calcium.

The experiment to be reported below was designed to measure the calcium excretion of an adult human on a low calcium diet, and to assess the value of the calcium in milk solids and in an inorganic salt, calcium gluconate, in replacing these losses and in maintaining a condition of calcium equilibrium. Since the human organism, perhaps to a greater extent than the lower animals, is subject to wide fluctuations in its utilization of dietary minerals, the experiment was planned to extend over much longer than the usual periods of time. A total of 168 days of experimental observation is included in this experiment.

PLAN OF EXPERIMENT

The subject of the experiment was one of the authors (F.R.S.), about 35 years of age, weighing normally about 85 kg., and 176.5 cm. high.

The plan of the experiment was to subsist throughout upon a low-calcium diet, upon which the daily loss of calcium in urine and feces would be determined. Then graded additions of calcium in the form of skim milk powder or of calcium gluconate were made to the diet and the calcium balance determined on each level in the attempt to find out how much calcium in the two forms would be required for calcium equilibrium. Such data would also permit the determination of the utilization of the calcium in each case by relating increments in calcium balance to increments in calcium intake.

The collection periods were of 4 days duration, though each experimental period of definite calcium intake lasted from 12 to 32 days. The feces of successive collection periods were separated with the aid of carmine markers. Each day samples of the foods consumed, in the proportions in which they were eaten, and equal in amount to one-fourth of the day's consumption, were collected, and every 4 days these samples were composited for analysis for calcium. Modifications of the McCrudden method were used in the analysis of food, feces and urine.

The low-calcium basal ration that was used throughout the experiment was as follows:

Breakfast	
Orange juice	100 cc.
Oatmeal	224 gm.
Lunch	
Bread	50 gm.
Bacon	48 gm.
Oatmeal cookies	60 gm.
Apples	180 gm.
Dinner	
Potatoes, baked	160 gm.
Beef	80 gm.
Beets	100 gm.
Prunes	120 gm.

The ratio of calcium to phosphorus in this day's menu was found by actual analysis to be 0.3 to 1. The day's intake of protein and energy in the basal diet amounted to about 64 gm. and 2627 calories, respectively, including the sugar and butter supplements given below.

Tea, made with distilled water, was taken with the noon and evening meals and all water drunk was distilled. During the day 94 gm. of sugar, partly in the form of candy, and 56 gm. of butter were also consumed. Vitamins A and D were provided daily in a vitamin capsule (Parke, Davis and Company) equivalent to 3 teaspoonfuls of cod liver oil. The skim milk powder supplements, ranging from 10 to 50 gm. daily, provided additional energy, as well as calcium, a circumstance that was considered favorable in view of the tendency for the body weight to decrease during the experimental periods. Following the intermission during the latter part of March and the first few days of April, and continuing throughout the calcium gluconate periods, the basal diet was supplemented with a further addition of 40 gm. of sugar daily in order to raise the energy intake to the level of the preceding periods when milk powder supplements were fed. The preparation of calcium gluconate used contained 9.084% of calcium.

The experiment consisted of forty-three 4-day periods, the sequence of which was interrupted only twice, from December 20th to January 6th and from March 23rd to April 7th. Throughout the period of experimental feeding the subject was in good health, and in fact felt better than usual.

EXPERIMENTAL RESULTS

The calcium balance data are summarized in table 1. The basal diet provided an average of 195 mg. of calcium daily, on which the average daily balances for the three basal periods were, in order, —99, —121 and —109 mg. daily, averaging —110 mg. The calcium excreted daily on this low-calcium diet averaged 304 mg., of which 68% was excreted by way of the intestinal tract. The close agreement of the metabolism

TABLE 1

The calcium metabolism data of the experiment

PERIOD	INCLUSIVE DATES	BODY WEIGHT	CALCIUM SUPPLEMENT CONSUMED DAILY	DAILY CALCIUM METABOLISM			
				Intake	Feces	Urine	Balance
		kg.		*gm.*	*gm.*	*gm.*	*gm.*
1[1]	Nov. 6–9	85.5	None	0.160	0.358	0.105	−0.303
2	Nov. 10–13		None	0.180	0.197	0.086	−0.103
3	Nov. 14–17		None	0.164	0.193	0.102	−0.131
4	Nov. 18–21	82.7	None	0.189	0.182	0.092	−0.085
5	Nov. 22–25		None	0.164	0.205	0.086	−0.127
6	Nov. 26–29		None	0.166	0.199	0.063	−0.096
7	Nov. 30–Dec. 3	80.8	None	0.228	0.204	0.070	−0.046
8	Dec. 4–7	81.0	None	0.198	0.226	0.072	−0.100
Averages		82.5		0.184	0.201	0.082	−0.099
9	Dec. 8–11	80.5	10 gm. milk solids	0.280	0.262	0.089	−0.071
10	Dec. 12–15		10 gm. milk solids	0.292	0.282	0.086	−0.076
11	Dec. 16–19	80.5	10 gm. milk solids	0.290	0.281	0.111	−0.102
Averages		80.5		0.287	0.275	0.095	−0.083
12[1]	Jan. 6–9		None	0.195	0.286	0.073	−0.164
13	Jan. 10–13		None	0.210	0.209	0.093	−0.092
14	Jan. 14–17		None	0.182	0.203	0.130	−0.151
Averages		...		0.196	0.206	0.111	−0.121
15	Jan. 18–21		40 gm. milk solids	0.692	0.575	0.140	−0.023
16	Jan. 22–25		40 gm. milk solids	0.709	0.584	0.127	−0.002
17	Jan. 26–29		40 gm. milk solids	0.716	0.650	0.118	−0.052
18	Jan. 30–Feb. 2	80.7	40 gm. milk solids	0.705	0.589	0.129	−0.013
19	Feb. 3–6	80.1	40 gm. milk solids	0.725	0.552	0.117	+0.056
Averages		80.4		0.709	0.590	0.126	−0.007
20	Feb. 7–10	80.3	50 gm. milk solids	0.832	0.755	0.189	−0.112
21	Feb. 11–14		50 gm. milk solids	0.856	0.652	0.163	+0.041
22	Feb. 15–18		50 gm. milk solids	0.856	0.751	0.143	−0.038
23	Feb. 19–22		50 gm. milk solids	0.850	0.697	0.136	+0.017
24	Feb. 23–26		50 gm. milk solids	0.868	0.677	0.144	+0.047
25	Feb. 27–Mar. 2		50 gm. milk solids	0.880	0.792	0.130	−0.042
Averages		80.3		0.857	0.721	0.151	−0.015
26	Mar. 3–6		40 gm. milk solids	0.718	0.582	0.126	+0.010
27	Mar. 7–10		40 gm. milk solids	0.726	0.609	0.121	−0.004
28	Mar. 11–14	79.8	40 gm. milk solids	0.745	0.605	0.126	+0.014
29	Mar. 15–18		40 gm. milk solids	0.721	0.634	0.096	−0.009
30	Mar. 19–22		40 gm. milk solids	0.758	0.647	0.147	−0.036
Averages				0.734	0.615	0.123	−0.004
31[1]	April 7–10	81.2	None	0.197	0.369	0.090	−0.262
32	April 11–14		None	0.209	0.214	0.098	−0.103
33	April 15–18	79.6	None	0.185	0.206	0.093	−0.114
34	April 19–22		None	0.196	0.215	0.106	−0.125
35	April 23–26	79.6	None	0.225	0.219	0.102	−0.096
Averages		80.1		0.204	0.213	0.100	−0.109
36	April 27–30		7.038 gm. Ca gluconate	0.816	0.640	0.160	+0.016
37	May 1–4		7.038 gm. Ca gluconate	0.884	0.600	0.186	+0.098
38	May 5–8	78.7	7.038 gm. Ca gluconate	0.796	0.577	0.172	+0.047
Averages		78.7		0.832	0.606	0.173	+0.053
39	May 9–12		5.304 gm. Ca gluconate	Collections lost			
40	May 13–16	78.9	5.304 gm. Ca gluconate	0.682	0.565	0.149	−0.032
41	May 17–20		5.304 gm. Ca gluconate	0.689	0.484	0.155	+0.050
42	May 21–24	78.2	5.304 gm. Ca gluconate	0.679	0.610	0.165	−0.096
43	May 25–28		5.304 gm. Ca gluconate	0.683	0.540	0.166	−0.023
Averages		78.5		0.683	0.550	0.159	−0.026

[1] The data for these periods are not included in the averages.

data in the three periods (November 6th to December 7th, January 6th to 17th, and April 7th to 26th) of subsistence on the basal diet alone is significant.

Forty grams of skim milk powder daily, providing about 542 mg. of calcium (1.355%) was sufficient in addition to the 195 mg. of calcium in the basal diet, to maintain the subject in approximate equilibrum, the average balance over a 20-day period being —7 mg. daily and over a subsequent 20-day period, —4 mg. daily. In the intervening 24-day experimental period, a supplement of 50 gm. of milk powder was no more effective than 40 gm. in maintaining calcium equilibrium, indicating the sufficiency of the latter supplement for calcium equilibrium.

For the 12 days from April 27th to May 8th, a daily supplement of calcium gluconate providing 639 mg. of calcium promoted some calcium storage averaging 53 mg. per day. During the following 20 days, this supplement was decreased to provide only 482 mg. of calcium daily. The contemporary balances of calcium were generally negative and averaged —26 mg. for the last 16 days for which balances were determined. The results indicate that 639 mg. of calcium added to the basal diet as the gluconate salt were more than adequate for the maintenance of calcium equilibrium, while a supplement of 482 mg. was inadequate. If interpolation is permissible on the basis of the observed balances of calcium, it appears that 534 mg. of added calcium as the gluconate would have established calcium equilibrium. This estimate is practically identical with the quantity of milk calcium found to be adequate—or only very slightly inadequate—for equilibrium, i.e., 542 mg.

DISCUSSION

The equation that Mitchell and Curzon ('39) derived to describe the average relationship in adult human subjects between the output of calcium and its intake is:

$$y = 0.6826\,x + 3.0940$$

where y is the calcium output in milligrams per kilogram of body weight per day and x is the intake of calcium expressed in the same fashion. In the experiments reported above, the average body weight of the subject in the three experimental periods in which no calcium supplement was added to the basal diet was 81.1 kg. His average daily intake of calcium per kilogram of body weight was therefore $195 \div 81.1 = 2.40$ mg. His average output on the same basis was $304 \div 81.1 = 3.75$ mg. Placing the former value for x in the above equation, gives a value for $y = 4.73$ mg., about 26% higher than the observed output, 3.75 mg. per kilogram. This divergence from the average relationship is perhaps not surprising in view of the disparity existing among the data from which the equation was derived. The coefficient of x in this equation indicates an average utilization of 32% ($[1 - 0.6826] \times 100 = 31.74$) of the dietary calcium.

About 540 mg. of calcium either in skim milk powder or in calcium gluconate seemed sufficient to attain calcium equilibrium in this subject in addition to the 195 mg. of calcium in the basal foods. Equilibrium was thus attained on a total of 735 mg. of calcium, equivalent to about 9.2 mg. per kilogram of body weight, as compared with the average of 9.75 mg. derived by Mitchell and Curzon ('39) from available data in the literature.

The daily addition of 540 mg. of calcium in the form either of milk solids or of calcium gluconate to the basal diet changed the daily calcium balance from an average of −110 mg. to approximately 0. In other words 540 mg. of calcium in these forms were required to replace an endogenous loss of 110 mg., indicating a utilization of the dietary calcium of about 20%.

This is a much lower utilization of milk calcium, or of dietary calcium of any origin, than is frequently assumed. It is difficult to find in the literature, however, any precise determinations of calcium utilization, since the information sought and obtained has been comparative in significance; for example, the relative utilization of the calcium of milk and of carrots. Such evidence as the following seems worthy of

citation. Mallon, Jordan and Johnson ('30) found that an intake of 9.6 mg. of calcium per kilogram of body weight daily, 94% of which was derived from milk, induced a small storage of calcium in one woman subject but a sizable loss of calcium in another. Mallon, Johnson and Darby ('32) reported that 9.3 mg. of calcium per kilogram of body weight per day of which 87 to 88% was supplied by milk was slightly inadequate for calcium equilibrium for two women subjects. In a study reported the following year ('33), the same investigators showed that a diet providing 9.8 and 10.5 mg. of calcium per kilogram of body weight daily, 88% of which was derived from milk, promoted a slight storage of the element in two female subjects. Using a group of ten college women as experimental subjects, Kramer, Potter and Gillum ('31) were unable to secure positive calcium balances on daily intakes of calcium ranging from 5.7 to 9.0 mg. per kilogram of body weight, 78 to 80% of the food calcium being derived from either milk or ice cream. These experimental results are consistent with the results reported in this paper, indicating a requirement of 9.2 mg. of calcium per day per kilogram of body weight for equilibrium, milk or calcium gluconate furnishing about 73% of the calcium consumed.

On the other hand, investigations by Adolph and Chen ('32), Rose and MacLeod ('23), Rose ('20) and Kramer, Latzke and Shaw ('28) indicate that diets providing daily from 8.2 to as low as 6.4 mg. of calcium per kilogram of body weight, supplied largely from milk, may promote calcium storage in adult human subjects for short periods of time, at least.

If these short-time calcium balance studies on adult subjects may be taken at their face value, they suggest that the utilization of calcium in any particular food, such as milk, is subject to considerable variation depending upon unknown factors, dietary or non-dietary or possibly both. It is not clear that the potential acidity or alkalinity of the diet, the proportion of fibrous materials in it, the proportion or character of the fat or mineral ratios are of preponderate importance at least when adequate amounts of vitamin D are available. The

possibility of raising the utilization of dietary calcium in the adult by varying the above (or other) factors is an important problem in human nutrition.

Turning to the calcium metabolism of children, it appears that, while the infant may utilize milk calcium to the extent of 60% or so (Mitchell and Curzon, '39), older children do not do so well. Sherman and Hawley ('22) placed three children, varying in age from 4 to 12 years, upon graded supplements of milk added to a low calcium diet. Comparing successive periods in these experiments, in which the milk intake was increased or decreased at the rate of 250 cc. daily, it may be shown that the increments or decrements in calcium retention average only 24% of the simultaneous increments or decrements in intake. Unpublished experiments of Outhouse, Kinsman and others at the University of Illinois on eleven preschool children afford excellent evidence of the efficiency in the utilization of milk calcium. Measuring the utilization of milk calcium by relating increments in calcium retention to corresponding increments in intake of milk calcium within ranges of the latter not exceeding the requirements for maximum retention, the percentages of utilization varied from 14 to 25 and averaged 20, exactly the same as the value secured on an adult reported in this paper.

SUMMARY

The calcium balance of an adult human subject receiving a basal low-calcium diet with varying supplements of calcium in milk solids and in calcium gluconate was followed throughout forty-three 4-day periods.

For equilibrium 9.2 mg. of calcium per kilogram of body weight per day were required, about three-fourths of which were supplied either in milk solids or in calcium gluconate. The calcium in both of these forms was equally well utilized, but only to the extent of 20%.

Grateful acknowledgment is made to Dr. T. S. Hamilton for advice concerning the chemical methods used in this investigation.

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THE LACTATION-PROMOTING EFFECT OF l-CYSTINE WHEN FED WITH ALFALFA PROTEINS [1]

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ONE FIGURE

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In a previous paper (Haag, '31) it was shown that the growth-promoting properties of alfalfa crude protein are improved by additions of l-cystine. This finding has since been confirmed and extended by Kellermann ('35). In the light of recent work dealing with the interchangeability of cystine and methionine, it appears that these and other cystine studies may need re-interpretation to the extent that cystine functions in supplementing a deficiency of methionine. More recently, Haag and Wright ('38) have reported preliminary studies in which the growth of nursing rats, as a measure of lactation, indicates that additions of l-cystine enhance the lactation-promoting properties of alfalfa crude protein. These studies are in harmony with much of our information dealing with the nutritive deficiencies of certain legume proteins and with recent (Daggs and Tomboulian, '35; Daggs and Lidfeldt, '38) work which has been interpreted as indicating that cystine may be looked upon as a dietary lactation stimulant. It is our purpose in this paper to present in detail some of our recent studies dealing with the influence of

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[2] The data reported in this paper are to be presented by L. D. Wright in partial fulfillment of the requirements for the degree of doctor of philosophy, Oregon State College.

l-cystine on lactation in rats fed on rations in which the principal source of nitrogen is obtained from commercial alfalfa leaf meal.

PROCEDURE

The general procedure has been to subject rats in advanced stages of pregnancy to a preliminary feeding period on a ration (no. 113) containing 50% of alfalfa leaf meal. As soon as young are born, the experimental ration (no. 114 or 115) is substituted for the preliminary ration. Daily records are kept of the weights of the female and of the litter of young. Not later than the second or third day the litter is reduced to six young. Feed intake records are kept on a weekly basis. Data are given for all females which completed a lactation period of 21 days with six young.

TABLE 1

Composition of rations used

INGREDIENTS	RATION NO.		
	113	114	115
Alfalfa leaf meal (2.99% N)	50.0	50.0	50.0
Starch	10.7	20.0	19.6
Sucrose	5.0	5.0	5.0
Hydrogenated fat	19.0	19.0	19.0
Cod liver oil	1.0	1.0	1.0
Yeast	3.0	3.0	3.0
$Na\ H_2PO_4 \cdot H_2O$	1.5	1.5	1.5
NaCl	0.5	0.5	0.5
Casein	9.0	...	...
l-cystine	0.3	...	0.4

RATIONS

The rations are of the same general type as those previously used (Haag, '31) in our growth studies. The composition of the rations used is given in table 1. The apparent digestibility of the dry matter in rations 114 and 115 is 72%; that of the crude protein 60%. The alfalfa leaf meal contained 0.37% total and 0.18% organic sulphur.

EXPERIMENTAL RESULTS

The results obtained are in part tabulated in table 2. These results include weights of the litters of six young for 0, 3, 17 and 21 days following birth, the day of birth being designated as the '0' day. The use of day 3 as a starting point permits postponing reducing the litter to six young and

TABLE 2

Live weight and feed consumption data on lactating females and weight of young

RAT NO.	DAILY FEED	WEIGHT OF FEMALES				WEIGHT OF SIX YOUNG			
		Days				Days			
		0	3	17	21	0	3	17	21
		Ration 114							
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
2057	18.3	229	217	162	157	32	39	81	85
2058	13.7	240	232	168	155	—	39	82	85
2060	17.6	297	280	198	184	36	50	84	96
2070	14.3	250	245	169	159	32	42	80	82
2161	12.2	332	309	220	208	34	43	86	88
2162	17.4	263	263	177	173	—	39	92	94
2300	16.9	265	258	187	183	36	45	96	110
2301	19.5	240	222	170	160	35	43	93	103
2303	16.2	220	215	160	154	29	37	75	79
Ave.	16.2	260	249	179	170	33	42	85	91
		Ration 115							
1864	23.1	320	312	267	248	37	55	142	171
2057	19.0	279	255	220	209	33	50	118	130
2058	17.9	245	235	206	198	35	44	112	125
2060	27.0	271	267	238	226	—	48	120	135
2061	20.7	285	284	—	239	35	55	—	157
2063	24.5	278	272	256	234	31	45	132	147
2145	17.4	282	270	231	220	35	48	126	147
2161	23.8	282	290	262	254	—	47	141	152
Ave.	21.7	280	273	240	229	34	49	127	146

the use of day 17 is justified on the basis that it approximately marks the end of the period when growth may be attributed to an exclusive milk diet.

In figure 1 are found the live weight curves for the litters of young rats. These curves are, with a few minor exceptions, plotted on a daily basis.

DISCUSSION

The graphic presentation in figure 1 emphasizes the striking character of the results obtained. The response to cystine feeding is marked and the uniformity of response to the respective rations is equally marked. The significance of the

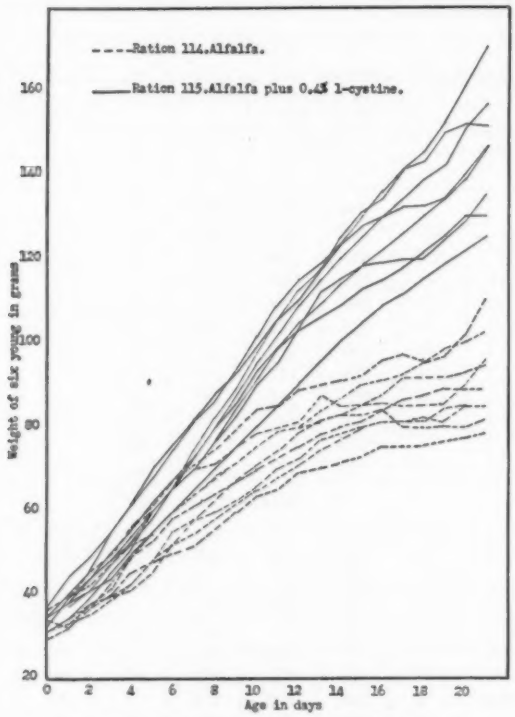

Fig. 1 Growth curves for litters of young.

response to cystine feeding, as reflected in the growth of the nursing young is enhanced by the effect of cystine in retarding the loss of weight by the lactating female. It will be noted that the cystine supplement usually increased the food intake, approximately doubled the rate of gain of the nursing young, and reduced the rate of body weight loss of the lactating

females approximately 50%. The average 'lactation index' values, as suggested by Daggs ('35), were 382 and 586, respectively, for rations 114 and 115.

In this group of experiments there was no predetermined plan for controlling the total food intake. During the first week of lactation the average food intakes on rations 114 and 115 were not significantly different. During the second and third weeks, however, the cystine supplemented ration was consumed far more freely.

This report would be incomplete without some reference to the ultimate significance of our results. Their application to practical alfalfa feeding problems is in part conditioned by the level (approximately 9%) of protein studied as compared to the 12 to 15% level commonly found in alfalfa hay. A most useful implication lies in the direction of developing more precise methods for evaluating proteins for their lactation-promoting properties.

The levels of alfalfa proteins at which cystine responses are effective, the response to various levels of cystine, the development of controlled-feeding technics analogous to the paired-feeding method, the response to other amino-acids, the determination of supplementary effects, and finally the determination as to whether the amino acid requirements for growth and lactation are of the same order, are questions which are now being investigated.

SUMMARY

The lactation-promoting properties of rations containing approximately 9% of alfalfa crude protein are markedly enhanced by 0.4% additions of l-cystine.

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THE DISTRIBUTION OF VITAMIN B_1 (THIAMIN) IN MEAT AND MEAT PRODUCTS [1]

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The value of meat and meat products as sources of protein, energy and minerals in the human dietary is generally recognized, but much less attention has been given to the vitamin content of these substances. With the increasing knowledge of the water soluble vitamins associated in the so-called B complex, it becomes more and more evident that meat and meat products cannot be ignored as important sources of many of these factors. The separation of the old factors into several vitamins and the recognition of new ones necessitates the reappraisal of many of the values now found in the literature.

The available information on the B complex is far from complete but at least six members, namely, thiamin, riboflavin, nicotinic acid, vitamin B_6, chick antidermatitis factor and factor W may be recognized as separate entities. There is need for much improvement in the methods used for the biological assay of these vitamins, but through the use of rats, chicks and dogs we have been able to make rather extensive studies on the distribution of these factors in meats. The studies on thiamin are reported in this paper.

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REVIEW OF THE LITERATURE

The investigations on the vitamin B content of meats made before 1923 were reviewed by Hoagland ('23 a). He ('23 b, '29) went on to study this question further by means of the pigeon protective method and found thereby a high content of this vitamin in pork muscle. Umishio ('30) determined the minimum amount of an alcoholic extract of raw and canned meat required for normal growth of rats on a B deficient ration. Roscoe ('31) studied this problem by means of the rat growth method. Christensen and co-workers ('36) studied the influence of cooking and canning on the vitamin B_1 content of lean beef and pork. Kellogg ('36) by means of a rat growth method found vitamin B_1 to be fairly evenly distributed in lamb tissues and organs. Plimmer et al. ('33) used pigeons in determining the antineuritic properties of meat. Baker and Wright ('35) used the bradycardia method on rats in their assays. Elvehjem et al. ('35) used chicks on an autoclaved ration in their studies. Recently Williams and Spies ('38) have summarized the vitamin B_1 content of animal tissues as determined by the various methods. The wide range of values for this vitamin in the same tissue gives ample proof for the necessity of further studies on vitamin B_1.

EXPERIMENTAL

The vitamin B_1 content of the tissues was determined by means of the rat growth method. The basal diet used was ration 112 described by Arnold and Elvehjem ('38 a) which has been shown to be complete in all respects except vitamin B_1. This ration was prepared as described by the above workers.

Each series was composed of about forty rats weighing between 35 and 45 gm. which were placed in individual screen bottom cages. All rats were kept on the basal ration for 1 week. Then a group of two rats, a male and a female, was placed on the supplemented basal ration containing a definite per cent of the tissue. In each series one group, which served as the negative control, was kept on the basal

ration, while the standard group received 80 micrograms of crystalline vitamin B_1 per 100 gm. of ration. The dried tissue was added to the basal ration at various levels in order to find the level which produced a gain in weight comparable to that of the standard group in that particular series.[2] All assays were made over a 6-week period during which time the rats were weighed weekly. The increases in body weight of the test and standard groups were compared and the vitamin B_1 potency calculated by means of a simple proportion.

The tissues used in these studies were from animals which had been slaughtered a day or so before shipment to the laboratory. All of the tissues were from animals which were graded at the time of slaughter as excellent.[3] As soon as the tissues arrived at our laboratory they were placed in a cold room after which they were prepared for drying.

Our first efforts were directed toward the development of a method for drying the tissues with as little destruction of vitamin B_1 as possible. Preliminary work indicated that tissues dried under vacuum at temperatures above 70°C. showed appreciable destruction of vitamin B_1. These tissues were prepared by separating the meat from as much fat and tendon as possible. The lean meat was then finely ground and spread out in thin layers (1 to 3 mm.) on pans over which a current of warm air (45 to 60°C.) was passed. The time required for drying has usually been 6 to 10 hours. The chemical analyses of these tissues as well as all other tissues assayed are given in table 1.

To determine whether any vitamin B_1 was destroyed during our drying process, both the fresh and the dried meat from the same animal were assayed simultaneously. The meat which was to be fed in the fresh condition was kept frozen

[2] The crystalline vitamin B_1 was accurately weighed out and made up to a definite dilution in N/10 HCl. This standard was added to a small part of the basal ration, dried in a vacuum desiccator and then mixed with a sufficient amount of the basal ration to produce a concentration of 80 micrograms of vitamin B_1 per 100 gm. of ration.

[3] We wish to thank Swift and Company, Armour and Company and Wilson and Company for furnishing samples of fresh tissues.

TABLE 1

Composition of animal tissues used in these studies

MEAT	MEAT PROCESS	MEAT SAMPLE	MOISTURE IN ORIGINAL SAMPLE	FAT IN DRIED SAMPLE	PROTEIN IN DRIED SAMPLE
			%	%	%
Beef brain	Dried	57	79.1	48.6	46.7
Beef heart	Dried	53	77.5	12.6	78.2
Beef heart	Stewed	54		11.3	81.4
Beef kidney	Dried	49	78.9	13.1	73.8
Beef kidney	Stewed	50		13.2	72.4
Beef liver	Dried	58	67.6	18.3	64.2
Beef lung	Dried	63	77.9	10.2	75.9
Beef pancreas	Dried	64	67.9	41.2	56.4
Beef round	Dried	40		16.6	75.6
Beef round	Broiled	41		23.1	73.0
Beef round	Fried	42		16.6	77.0
Beef round	Roasted	43		15.2	78.6
Beef spleen	Dried	59	76.8	12.8	77.4
Beef tongue	Dried	67	68.4	41.7	54.5
Veal hindquarter	Dried	44		15.8	79.2
Veal hindquarter	Fried	45		14.8	77.5
Veal hindquarter	Roasted	46		4.2	83.0
Veal liver	Dried	70		20.2	65.2
Veal, leg of	Dried	56	76.4	12.4	81.5
Lamb liver	Dried	61	65.5	23.5	57.3
Lamb, leg of	Dried	35		22.2	72.6
Lamb, leg of	Dried	55	72.3	21.3	74.1
Pork ham	Dried	32	72.5	19.0	76.2
Pork ham	Dried	51	74.5	21.2	74.2
Pork ham	Fried	52		20.4	76.5
Smoked ham	Dried	47	67.5	14.4	63.1
Smoked ham	Fried	48		16.0	62.6
Canned ham	Dried	72	68.7	10.8	67.6
Pork heart	Dried	71		14.4	70.4
Pork kidney	Dried	62	78.4	18.6	72.8
Pork liver	Dried	33	70.5	13.8	55.8
Pork loin	Dried	34	71.5	24.3	57.4
Pork loin	Dried	36		31.0	54.5
Pork loin	Baked	37		34.4	59.3
Pork loin	Fried	38		28.4	62.8
Pork loin	Roasted	39		30.8	61.3
Poultry, dark	Dried	66	74.2	18.8	75.2
Poultry, dark	Dried	69	76.4	12.2	80.4
Poultry, light	Dried	65	72.8	11.6	84.4
Poultry, light	Dried	68	75.4	5.4	83.9

in the cooler till needed. Daily food consumption records were kept on all rats receiving various levels of pork ham, pork loin and pork liver. In another series the rats received the fresh tissues in daily supplements equivalent to the dry tissues consumed by the rats in the corresponding group in the first series. The rats in the second series had free access at all times to the basal ration. These results (table 2) show that the dried pork ham (sample 33) contained 11 I.U.[4] of vitamin B_1, which agrees well with 10 I.U. per gram (on the dry weight basis) for the fresh tissue. The values of the vitamin B_1 content of the fresh and dried pork loin and liver are within experimental error. This agreement warrants the conclusion that very little if any vitamin B_1 is destroyed by our method of drying.

We therefore proceeded to collect, dry and assay a variety of meats and meat products. The results show an exceedingly high content of vitamin B_1 in pork muscle. The pork loin (sample 34) used in the above studies contained 13 I.U. per gram. It was from an animal killed in September. Another sample of pork loin (sample 36) from an animal slaughtered in November contained 20 I.U. of vitamin B_1 per gram. A similar variation was noted in some samples of pork ham. One sample secured in September (sample 33) from the same animal as the first pork loin (sample 34) contained 11 I.U. per gram of dried material. A second sample of ham (sample 52) was secured in January of the following year from a different source. This sample had 20 I.U. of vitamin B_1 per gram of dried material.

The values for the vitamin B_1 content of pork muscle are considerably higher than those of other meats. Beef round contained 3 I.U. of vitamin B_1 per gram and two samples of leg of lamb contained 4 I.U. of vitamin B_1 per gram. One sample of leg of veal secured in December contained 4.5 I.U. of vitamin B_1 per gram whereas a later sample secured in March contained 1.6 I.U. per gram. Excluding

[4] The conversion factor 1 I.U. = 3 micrograms of crystalline vitamin B_1 was used (Arnold and Elvehjem, '38 b).

TABLE 2

The vitamin B_1 content of meat and meat products

MEAT	MEAT SAMPLE	MEAT PROCESS	EXPERIMENT NO.	VITAMIN B_1 CONTENT	
				γ per gram [1]	I.U. per gram [1]
Beef brain	57	Dried	1883	8	2.7
Beef heart	53	Dried	1854	30	10
Beef heart	54	Stewed	1857	13	4.5
Beef kidney	49	Dried	1894	15	5
Beef kidney	50	Stewed	1895	9	3
Beef liver	58	Dried	2827	12	4
Beef lung	63	Dried	2810	9	3
Beef pancreas	64	Dried	2811	10	3.3
Beef round	40	Dried	1826	9	3
Beef round	41	Broiled	1858	4.5	1.5
Beef round	43	Roasted	1872	4 [2]	1.3 [2]
Beef round	42	Fried	1832	9	3
Beef spleen	59	Dried	1887	7.0	2.5
Beef tongue	67	Dried	2824	9	3
Lamb liver	61	Dried	2826	12	4
Lamb, leg of	35	Dried	884	12	4
Lamb, leg of	55	Dried	1876	12	4
Veal hindquarter	44	Dried	1834	13.5	4.5
Veal hindquarter	56	Dried	2844	5.2	1.6
Veal hindquarter	45	Fried	1836	7.5	2.5
Veal hindquarter	46	Roasted	1874	6	2
Veal liver	70	Dried	2845	5.2 [2]	1.7 [2]
Pork ham	32	Dried	1805	33	11
Pork ham	32	Fresh	1813	30	10
Pork ham	51	Dried	1850	60	20
Pork ham	52	Fried	1852	60	20
Smoked ham	47	Dried	1843	33	11
Smoked ham	48	Fried	1845	29.0	9.5
Canned ham	72	Dried	2846	26	8.6
Pork heart	71	Dried	2848	25.5	8
Pork kidney	62	Dried	1893	24	8
Pork liver	33	Dried	875	16	5.3
Pork liver	33	Fresh	876	15	5
Pork loin	34	Dried	1807	39	13
Pork loin	34	Fresh	1815	39	13
Pork loin	36	Dried	897	60	20
Pork loin	37	Baked	891	30	10
Pork loin	38	Fried	893	39	13
Pork loin	39	Roasted	895	30	10
Poultry, dark	66	Dried	2816	9	3
Poultry, dark	69	Dried	2837	7.6	2.5
Poultry, light	65	Dried	2839	<5.5	<1.8
Poultry, light	68	Dried	2836	3 [2]	1 [2]

[1] On the dry weight basis.

[2] Calculated—the level of tissue could not be fed high enough for optimal growth.

veal, the livers of the other animals were remarkably constant in their vitamin B_1 potency in spite of rather wide fluctuations in the content of this vitamin in their muscles. The livers from beef, hog and lamb contained approximately the same amount of vitamin B_1, 4 to 5.3 I.U. per gram of dried material. In contradistinction to these values was that of veal liver which contained only 1.7 I.U. of vitamin B_1 per gram. This sample was a composite of three calves which were slaughtered in July.

Beef heart was a very good source of vitamin B_1. Our sample, which was a composite from eight different animals, contained 10 I.U. of vitamin B_1 per gram of dried material. Pork heart contained slightly less than this or 8 I.U. of vitamin B_1 per gram. There were 8 I.U. in each gram of pork kidney whereas beef kidney had 5 I.U. per gram. In both of these cases the kidney was richer in vitamin B_1 than the liver of the same animal. Beef lung and beef tongue both contained 3 I.U. of vitamin B_1 per gram, making them as potent as the muscles from these animals. In the case of beef brain, the gray matter was separated from the white and only the white matter was used. According to our assays there were 2.7 I.U. of vitamin B_1 per gram of dried brain. Our values for beef kidney, beef lung and beef spleen agree well with those reported by Arnold and Elvehjem ('38 c) who used the chick growth method of assay. They found these tissues to contain 5, 2 and 2 I.U. per gram of dried tissue respectively.

The first samples of poultry which we assayed were from two cocks which were nearly 3 years old. The dark meat on the lower legs and abdomen and the light meat on the breast and wings were prepared separately. The second samples of poultry were from fifteen pullets about 5 months old. In both cases the assays for vitamin B_1 indicated a higher content of the vitamin in the dark meat than in the light. The dark meat from the older birds contained 3 I.U. of vitamin B_1 per gram of dried material while that from the younger animals contained 2.5 I.U. per gram. The light meat in the older birds contained less than 1.8 I.U. of vitamin B_1 per

gram and that from the younger animals contained about 1 I.U. per gram.

The samples which were used for cooking were cuts of meat such as are sold commercially. They were prepared according to the directions which have been described by Child ('38). In each case a part of the uncooked sample was dried according to our method and this was used in determining the original vitamin B_1 content of the tissue. The cooked and uncooked meats were assayed simultaneously for

TABLE 3

Stability of vitamin B_1 during household cooking processes

PROCESS	MEAT	MEAT SAMPLE	TIME OF PROCESSING	DESTRUCTION OF VITAMIN B_1
Frying	Beef round	42	20 minutes	0
	Veal hindquarter	45	20 minutes	45%
	Pork ham	52	15 minutes	0
	Smoked ham	48	15 minutes	10%
	Pork loin	38	Medium well done	35%
Roasting	Beef round	43	2.5 hours	61%
	Veal hindquarter	45	2 hours	58%
	Pork loin	39	1.5 hours	50%
Broiling	Beef round	41	20 minutes	50%
Baking	Pork loin	37	1 hour	50%
Stewing	Beef heart	54	1 hour	55%
	Beef kidney	50	45 minutes	40%

vitamin B_1 by the rat growth method. From the difference in the vitamin B_1 content of these samples the amount of destruction of the vitamin was calculated (table 3). These results indicate that the stability of vitamin B_1 in meats during cooking depended to a certain extent upon the method of cooking. Frying apparently produced the least change in the antineuritic vitamin as shown by the almost complete preservation of the vitamin in fried beef round and fried pork ham. When smoked ham was fried some 10% of the

vitamin was destroyed. This was increased to 35% in the case of pork loin and 45% in veal chops.

Roasting seemed to have a greater destructive action on the vitamin B_1 in the three samples tested, for this method of cooking inactivated from 50 to 60% of the vitamin. The beef round, veal hindquarter and pork loin showed a greater preservation of the vitamin with a decrease in the time of roasting. The broiled beef round and baked pork loin both showed a 50% destruction of the vitamin B_1 content after cooking. The stewed beef heart and stewed beef kidney showed a stability of the vitamin similar to that of the broiled meat. The rather marked destruction of the vitamin produced by roasting, broiling, baking and stewing is possibly associated with the long time and high temperature used in cooking.

Since several workers, Evans and Lepkovsky ('29), Salmon and Goodman ('37), Stirn and Arnold ('38) have shown that fat decreases the vitamin B_1 requirement of rats, we determined whether the fat content of these tissues had any influence on our biological assays. A series of rats was fed various levels of coconut oil and suboptimal amounts of vitamin B_1 as supplements to our basal ration. By this means it was found that the rats receiving added fat in their ration up to levels of 20% grew no better than those getting only the corresponding amount of vitamin B_1. The level of tissue required for an assay has usually been less than 10%. This definitely indicates that the fat in the tissues assayed had no influence on the result of these experiments.

DISCUSSION

In any study on the quantitative distribution of specific nutrients, the figures have little significance unless they are related to human requirements. This paper indicates that our meat consumption is able to supply a considerable fraction of our daily vitamin B_1 requirement.

Investigations on the vitamin B_1 content of our daily foods have shown that most of these substances contain from 0.5

to 2.0 I.U. of vitamin B_1 per gram. Our work shows that meats are above the average in this respect and compare favorably with many foods which are ordinarily considered potent sources of this vitamin. For instance, Baker and Wright ('35) found different samples of whole wheat to contain from 2.3 to 3.4 I.U. of vitamin B_1 per gram while our values for meats show them to contain 2 or more I.U. per gram. Even after cooking, meats are still within the above range of vitamin potency.

Although there are no accurate figures indicating the amount of meat used in the average American diet, the figures of Stiebling and Ward ('33) indicate that the typical diet contains about 7% of meat. The average daily food consumption would have to approximate 700 gm. in order to supply a daily energy requirement of about 2700 Calories. Since about 7% of the daily food is meat, then this constituent will supply not less than 150 I.U. of vitamin B_1 per day (on the basis that meat contains 3 I.U. of vitamin B_1 per gram). A confirmation of this value is obtained by calculating the amount of vitamin B_1 in the yearly meat consumption of city wage earners. According to calculations based on the most recent statistics of this sort (Stiebling, '38), an average person secures over 200 I.U. of vitamin B_1 each day from his meat intake.

The daily human requirement for vitamin B_1 has been placed at 200 to 500 I.U. (Williams and Spies, '38). Therefore, even though meats are used in the diet to the extent of only 7%, they are capable of supplying one-third of the human vitamin B_1 needs. When meat such as pork is used, the proportion of the day's requirement thus supplied is correspondingly increased.

A pork chop, even when fried, may supply the total daily requirement of this vitamin. An ordinary pork chop contains approximately 25 gm. of dried meat. Our sample of fried pork loin (sample 38) contained 13 I.U. of vitamin B_1 per gram which means that there may be 325 I.U. of vitamin B_1 in a pork chop.

SUMMARY

1. A biological method of assaying various materials for their vitamin B_1 content has been described. This is based on a comparison of the growth of rats on a basal ration containing the material to be assayed with the growth of rats on the basal ration with added amounts of crystalline vitamin B_1.

2. A simple method of drying moist materials, which minimizes destruction of vitamin B_1, has also been described.

3. By our method of assay a large number of meats and meat products have been tested for their vitamin B_1 content. A study has also been made on the stability of the vitamin under various types of household cooking. Here it was found that there was slight destruction of the vitamin during frying, but with roasting, broiling or stewing, the destruction of the vitamin approached 50%.

4. A study of the influence of fats on the growth of the rats on our basal ration has been made. Our work showed that levels of fat up to 20% had no influence on the validity of our assays.

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THE REQUIREMENTS OF THE DOG AND THE RAT FOR NICOTINIC ACID

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THREE FIGURES

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I. THE MINIMUM AMOUNT REQUIRED BY THE DOG

The work of Elvehjem et al. ('37, '38) has made it evident that nicotinic acid and nicotinic acid amide are effective in curing the deficiency disease known as canine blacktongue. Other workers, Street and Cowgill ('37), Smith et al. ('38), have confirmed this observation. Fouts et al. ('37) have shown that human pellagra may be cured by feeding 500 mg. of nicotinic acid per day. Spies et al. ('37) also found that the mucous membrane lesions of pellagrins could be cured by feeding nicotinic acid. Chick et al. ('38) fed 60 mg. nicotinic acid per day to swine on pellagra producing diet and found that it induced a normal growth rate and maintained the animals in good condition.

The minimum need for nicotinic acid has not, however, been determined for any species. Elvehjem et al. ('37, '38) were able to cure acute blacktongue with a single dose of 25 or 30 mg. of nicotinic acid feeding 100 mg. per week subsequently prevented the reappearance of symptoms. Street and Cowgill ('37) obtained good growth with dogs by feeding 25 or 50 mg. per dog per day, while Smith et al. ('38) obtained growth with 1.0 mg. nicotinic acid per day per kilogram body

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weight. The effect of lower doses has not been reported except for a recent publication by Sebrell et al. ('38) which is referred to later.

The following experiments were, therefore, undertaken with the object of determining the minimum daily requirements of the dog for nicotinic acid.

EXPERIMENTAL

The ainmals used in these experiments were adult dogs of mixed breed, weighing 5 to 10 kg. The diet consisted of:

	%
Yellow cornmeal	74.0
Pea meal	8.0
Casein (alcohol extracted)	9.0
Cottonseed oil	6.0
Sodium chloride	0.5
Calcium carbonate	1.0
Calcium phosphate	1.0
Iron oxide	0.04

The diet was cooked fresh each day by adding water to it and steaming in a steam cooker for 2 hours; 1% of cod liver oil was mixed in the diet after cooking. Synthetic thiamin and pure crystalline riboflavin were fed at levels of 0.1 mg. and 0.5 mg. per dog per day respectively.

A combination of the curative and protective methods have been used for assaying the minimum amount of nicotinic acid necessary. In the earliest experiments graded daily doses of nicotinic acid were fed to the animals after they had been on the deficient diet for several weeks, but had no definite symptoms of blacktongue. It was found that the lowest amount tried 2.5 mg. per day produced a rapid increase in weight and complete protection (fig. 1).

For assaying smaller amounts a slightly different method was used. The animals were first run out of nicotinic acid until they showed definite symptoms of blacktongue. The symptoms were then cured by injecting 10 mg. of nicotinic acid on 3 successive days. The amount of nicotinic acid was then reduced to the lower levels of 0.25, 0.5 and 1.0 mg. per

day which was given by mouth. Blacktongue symptoms again developed when 0.25 and 0.5 mg. were fed; with 1.0 mg., however, complete protection and slow increase in weight resulted over a period of 12 weeks. These results are shown in figure 1.

In later experiments attempts were made to cure animals with blacktongue by giving 1.0 mg. of nicotinic acid per day, without first giving the 30 mg. dose. When the symptoms were well marked this method always failed to bring about a cure. If, however, the 1.0 mg. dose was started before

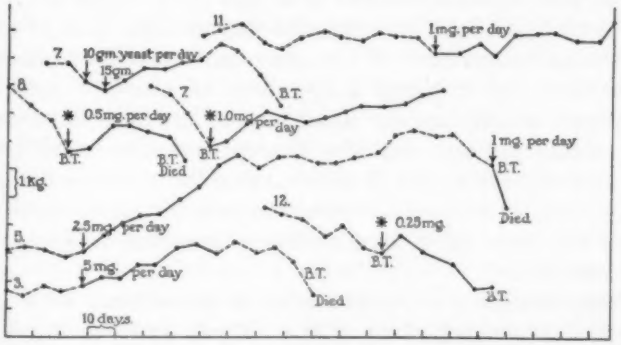

Fig. 1 Growth curves of dogs fed Goldberger diet. Broken lines indicate that the deficient diet alone was being fed. Continuous lines indicate that a supplement of either yeast or nicotinic acid was given. * = 30 mg. of nicotinic acid was injected in three successive daily doses of 10 mg.

severe symptoms had developed the animals would be protected and commence gaining in weight slowly. These results are shown in figure 1 (animals 5 and 11). It was found necessary to feed 15 gm. of yeast per day to the positive controls in order to get a steady increase in weight. The minimum protective dose of yeast may, however, be smaller than this.

DISCUSSION

It is evident from these experiments that the minimum dose of nicotinic acid necessary to protect the dog against blacktongue is about 1.0 mg. per day. When the results are

worked out on the basis of milligrams of nicotinic acid fed per kilogram of body weight it is found that 0.25 mg. per kilogram gave rapid increase in weight and complete protection; 0.13 mg. per kilogram gave complete protection and a slow increase in weight; while 0.084 and 0.027 mg. per kilogram gave no protection.

Considerable variation in the time which dogs take to develop blacktongue after being changed from an adequate diet to the deficient one has been noted. Thus dog no. 3 which had been having 5 mg. of nicotinic acid developed blacktongue in 5 weeks after the dose had been stopped. While no. 5 which had been receiving 2.5 mg. per day took 13 weeks to develop symptoms. When dogs have just been cured of blacktongue by a single large dose of nicotinic acid, the symptoms quickly appear again unless the dose is repeated. This would indicate that the general nutritive condition of the animal plays a part in determining the time for the onset of the disease, rather than the actual amount of nicotinic acid which the dogs have been receiving previous to giving the deficient diet.

These results are substantially in agreement with those reported by Sebrell et al. ('38). These authors found that when a semi-weekly dose of 1 mg. nicotinic acid, equivalent to 0.28 mg. per day was given, blacktongue developed. When the equivalent of 0.85 mg. per day was fed, some symptoms of incipient blacktongue appeared, but with higher doses complete protection was maintained. They also found that the curative method gave highly irregular results. The amount of nicotinic acid necessary to cause the symptoms of blacktongue to subside appeared to be between 20 and 60 mg. The duration of the cure, however, was not strictly proportional to the dose given and large variations were encountered.

It is interesting to compare the minimum protective dose of nicotinic acid for the dog with that of yeast found by Goldberger et al. ('28). These workers found that when 1.8% of yeast was mixed with the blacktongue producing

diet, almost complete protection was observed over a period of more than 300 days. This percentage appears to be roughly equivalent to 4 gm. of yeast per day. In curative experiments they found it necessary to feed 20 gm. of yeast per day to a 10 kg. dog to obtain a cure. Afterward 10 gm. of yeast per dog were required to prevent a recurrence of symptoms. This is approximately the same amount as was used here for the positive controls. Thus it would appear that 1.0 mg. of nicotinic acid is contained in 4 to 10 gm. of yeast.

Goldberger and Tanner ('25) found that 50 gm. of yeast per day was sufficient to cure pellagra encountered among colored females at the Georgia State Sanatorium. This would suggest, therefore, that 5 to 12 mg. of nicotinic acid should be sufficient to cure human pellagra. This amount is much smaller than that used by Fouts et al. ('37) and Spies et al. ('37) who fed as much as 500 to 1000 mg. per dag.

A recent observation by Schmidt and Sydenstricker ('38) has shown that when 90 gm. of yeast per day were fed to a group of pellagrins, a marked improvement in their condition occurred. When 100 mg. of nicotinic acid were given twice weekly to another group some improvement was noted at first, but it was not maintained. At the end of a 6-week period their condition was little better than it was before treatment.

These workers suggest that 90 gm. of yeast are equivalent to 15 mg. of nicotinic acid, which is in agreement with the calculation made here. From these observations it would seem that yeast is more effective in the treatment of pellagra than is the equivalent amount of nicotinic acid calculated on the basis of experiments with dogs.

It must be pointed out, however, that in Schmidt and Sydenstricker's experiments the diet of the pellagrins was not strictly controlled and may have been deficient in other factors such as riboflavin. These factors would be supplied by yeast and may help in bringing about a complete recovery.

II. IS NICOTINIC ACID AN ESSENTIAL SUBSTANCE FOR THE RAT?

It is still uncertain whether nicotinic acid is an essential substance for the rat or not. Rhodes and Miller ('35); Birch, Gyorgy and Harris ('35); Birch, Chick and Martin ('37) found that rats fed on pellagra producing diets were able to thrive, whereas dogs or swine reared on a similar diet rapidly succumbed. Since it has been shown that nicotinic acid is the substance missing from the pellagra producing diet, it would be presumed that rats do not need this substance. Other workers, however, have presented evidence which indicates that this substance is necessary for the rat. Euler and Malmberg ('36) fed rats on a basal vitamin B-free diet, supplemented by an acid clay adsorbate for vitamin B_1, riboflavin and an acid clay filtrate prepared from yeast, for other B vitamins. Over half of the experimental animals on this diet died, the remainder showed 'irregular' gains in weight, averaging 0.9 gm. per day. When 1.0 mg. of nicotinic acid amide was fed per day, only one animal out of nine died, the rest showed 'regular' gains in weight. Funk and Funk ('37) state that pigeons and rats fed on purified food ingredients plus thiamin, cod liver oil and vitamin B complex (U. S. Vit. Corp.) gave larger food intakes and better weight gains when nicotinic acid or the amide were fed than did the controls which received no nicotinic acid. Frost and Elvehjem ('37) obtained a definite growth response when nicotinic acid and adenine nucleotides were fed to rats on a vitamin B-deficient diet supplemented by thiamin and riboflavin. A dose of 1.0 mg. per day was necessary in order to produce a maximum effect.

More recently Gyorgy ('38) showed that the inclusion of 0.1% nicotinic acid in the diet of vitamin B_6 deficient rats prevented the appearance of panmyelophthisis described by Gyorgy et al. ('37). In view of this conflicting evidence it was thought desirable to reinvestigate the question as to whether nicotinic acid is essential for the rat or not.

The difficulty in such experiments is to supply the other factor in the vitamin B_2 complex free from nicotinic acid.

Previously maize has been given as a source of vitamin B_2 complex. The feeding of this foodstuff, however, raises the possibility that the animals may develop 'refection' due to the starch present in the maize and so synthesize nicotinic acid, in their intestinal tracts by bacterial action.

In these experiments, therefore, the use of starch has been avoided by feeding the following diet:

	%
Alcohol extracted casein	20
Lard	20
Sucrose	55
Salts	4
C.I.O.	1

The inclusion of this amount of lard in the ration delays the onset of vitamin B_6 deficiency (Birch, '38), so that one is able to keep animals alive on this diet for a long period when it is supplemented only by thiamin and riboflavin. Lard apparently has no sparing action on nicotinic acid deficiency as it has been repeatedly used in pellagra producing diets, Goldberger et al. ('28), Ruffin and Smith ('34).

This diet should be absolutely free from nicotinic acid and although deficient in other members of the vitamin B_2 complex, it was hoped that some symptoms of nicotinic acid deficiency, such as the panmyelophthisis described by Gyorgy et al. ('37) would show up before the animals died from other deficiencies. Nicotinic acid was fed to some of the animals at levels of 1.0 and 0.1 mg. per day. No significant difference was, however, seen between those receiving nicotinic acid and those not. Typical growth curves of some of the animals are shown in figure 2. The symptoms exhibited at death were: a sparse greasy fur which was covered with dried blood due to hemorrhage from the edges of the ears and the nose; acrodynia was seen in over 50% of the animals; respiratory infections were also a frequent cause of death.

These experiments show that complete deficiency of nicotinic acid in the rat for over 150 days is not accompanied by any specific physiological dysfunction such as is seen in dogs, swine or in man. They do not, however, exclude the pos-

sibility that nicotinic acid may be necessary for normal growth. This latter possibility is rendered unlikely by the finding that good growth may be obtained with rats on a vitamin B_2 complex deficient diet by feeding molasses (fig. 3). This material is apparently free from nicotinic acid as it has been used extensively in pellagra producing diets by Goldberger and Tanner ('25) and by Ruffin and Smith ('34).

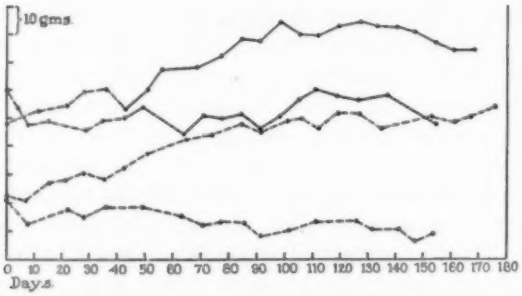

Fig. 2 Growth curves of rats on basal vitamin B-free diet containing 20% lard, supplemented by thiamin and riboflavin. Continuous lines indicate that nicotinic acid was also fed.

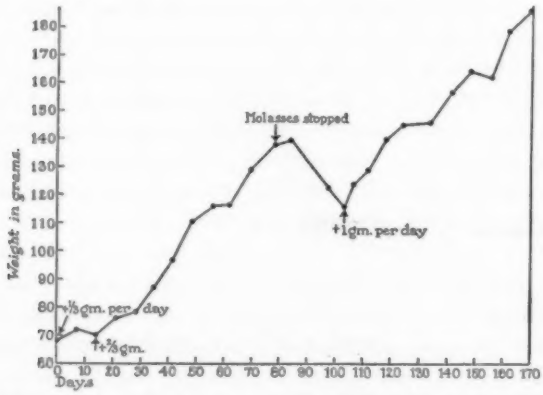

Fig. 3 Growth curve of rat on a diet consisting of casein 20%, sucrose 66%, butterfat 9%, salt mixture 4%, cod liver oil 1%, supplemented by thiamin and riboflavin. Molasses was fed in amounts indicated when the animal had developed acrodynia.

DISCUSSION

The most significant evidence that nicotinic acid or the amide are important factors in the nutrition of the rat is afforded by the work of Frost and Elvehjem who obtained a growth response of 20 gm. per week for 5 weeks when 1.0 mg. of nicotinic acid amide was fed per day. These workers showed that their factor W could be replaced by a mixture of nicotinic acid amide and adenine nucleotides. The subject is complicated, however, by the previous finding of Elvehjem, Koen and Oleson ('36) that factor W was different from the anti-blacktongue factor (now known to be nicotinic acid) and that it was thermolabile. Furthermore, when nicotinic acid amide was fed at the level of 80 micrograms per day (equivalent to 0.8 mg. per kilogram body weight) only a slight stimulation of growth ensued. Considering that 0.13 mg. per kilogram is sufficient to show a definite response in the dog which is extremely sensitive to nicotinic acid deficiency, 0.80 mg. per kilogram should be more than enough to produce a maximum effect in the rat if this substance were essential. It is, therefore, suggested that an interpretation different from that which involves the assumption that nicotinic acid is an essential substance for the rat may be used to explain the results of Frost and Elvehjem. For instance it is possible that the animals developed refection as 12% uncooked maize is fed in their diets.

With regard to the panmyelophthisis which Gyorgy ('38) is able to prevent by feeding nicotinic acid, it must be remembered, as Gyorgy himself pointed out, that no cures of this disease have been demonstrated. The present author has failed to produce this disease, and it is believed that other conditions which are not yet fully understood influence its appearance.

Details of Funk and Funk's work have not yet appeared and so it is therefore difficult to evaluate their findings. It is worthy of mention in connection with their work, that recently Carter and O'Brien ('38) have found that maize forms a complete diet for pigeons and addition of nicotinic acid had no weight restorative effect.

It appears, therefore, that the evidence in favor of the assumption that nicotinic acid is necessary for the rat is very inconclusive. In view of the failure of these present experiments to find any support for such a conclusion, it is reasonable to suppose that nicotinic acid is not necessary until more definite evidence is obtained for either view.

Attempts to estimate nicotinic acid by the dinitro chlorobenzene reagent used by Vilter et al. ('38) gave promising results when pure nicotinic acid was used, but the method was inapplicable to tissue extracts owing to impurities also giving colors which obscured that given by nicotinic acid. It was, therefore, impossible to determine if the tissues of rats on the nicotinic acid free diet contained this substance in normal amounts.

SUMMARY

PART I

The minimum dose of nicotinic acid necessary to protect the dog against blacktongue was found to be about 1.0 mg. per day for an animal of 5 to 10 kg. On a body weight basis 0.25 mg. of nicotinic acid per kilogram gave a rapid increase in weight and complete protection, 0.13 mg. per kilogram gave complete protection and a slight increase in weight, while 0.084 and 0.027 mg. per kilogram gave no protection. It has been calculated that 1.0 mg. of nicotinic acid is contained in 4 to 10 gm. of yeast.

PART II

Evidence is presented which indicates that nicotinic acid is not an essential substance for the rat.

I have pleasure in expressing my thanks to Dr. J. T. Wearn for the facilities afforded for this work, and to the S. M. A. Corporation and Messrs. Merck & Co. for gifts of riboflavin. The work was financed by the Bourne Fund in the department of medicine of Lakeside Hospital.

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THE ENERGY CONTENT OF GOAT MILK [1]

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THREE FIGURES

(Received for publication November 7, 1938)

The purpose of this work was to study the correlation between energy value and some constituents of milk with the object of establishing ways of calculating the energy value instead of determining it directly.

The energy content of milk is important as an index of the food value of this constituent of the diet. The nutritive value of milk lies chiefly in its content of protein and energy not to mention the mineral matter and vitamins. From the standpoint of the efficiency of the milk goat as a transformer of food energy into milk energy, it is necessary to know what per cent of the food energy utilized appears as milk energy.

The experiments reported by Graham et al. ('38) show that the efficiency of the mammary gland is more than 90%. The calculations included the maintenance energy of the mammary tissue as well as the cost of the production of the milk and would therefore show that the cost of transference of blood precursors to milk constituents would be less than 10%.

The calorific value of cow's milk has been determined by a number of investigators. Overman and Sanmann ('26) determined the energy content of 212 samples of cows' milk. They found the correlation between fat percentage and calories per kilogram of milk to be $r = 0.9814 \pm 0.0017$, indicating that the energy content of the milk could be estimated with a high degree of accuracy from its weight and fat percentage.

[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 582.

Overman, Sanmann and Wright ('29) reported the analysis of 1999 samples of milk. These data were studies by Overman and Gaines ('33) in conjunction with the 212 analyses of milk for energy residing in the various components of milk as follows in calories per gram: fat 9.312, protein 5.358, lactose 3.987, ash 4.980 and water —0.036. They did not explain the energy calculated to reside in the ash. They are of the opinion that all of the constituents should be considered and computed from the following formula.

$$E = 93.12f + 53.58p + 39.87l + 49.80a - 0.356w$$

where E is calories per kilogram of milk and f, p, l, a and w are the percentages of fat, protein, lactose, ash and water, respectively. These values vary to some extent depending on the number of components studied in calculating the values. The standard values reported by Abderhalden ('08) are fat 9.23, protein 5.71 and lactose 3.95 calories per gram. The value obtained by Overman and Gaines for fat agrees closely with the standard value, which is probably due to the greater proportion of the total energy being represented by fat and also to the accuracy of the fat determination.

During the course of experiments on the nutrition of the mammary gland of the goat, eighteen determinations of the energy value of the milk were made and compared with the values obtained by calculating the energy content from the composition of the milk. These values were compared with those of cow's milk of corresponding fat percentage.

The eighteen milk samples studied were obtained from four goats. Samples were taken at all stages during the normal lactation period from two of the goats.

Determinations of fat, total nitrogen, lactose, inorganic and total phosphorus, total solids and energy value were made for nearly all of the samples studied. The determinations were made as soon as the sample of milk was obtained and preservatives were not used. All of the determinations were made in duplicate with the exception of the energy determinations which were run in triplicate.

The percentage of fat was determined by the Babcock method. Total nitrogen was determined by the official Kjeldahl method (1934) and milk protein was obtained by multiplying the total nitrogen by 6.38. Total solids were determined in the usual way and the dried sample used for the determination of energy value (table 1).

TABLE 1

The composition of goat milk—by determination

GOAT NO.	NITROGEN	LACTOSE	FATS	SOLIDS	ENERGY PER 100 ML. OF MILK	ENERGY PER GRAM OF DRIED MILK	TOTAL PHOSPHORUS MILLIGRAM PER 100 ML.	INORGANIC PHOSPHORUS MILLIGRAM PER 100 ML.
	%	%	%	%				
348	0.59	6.02	4.80	19.12	114.00	5.96	.....	62.76
348	0.65	5.24	5.25	16.11	94.04	5.84	.....	63.98
348	....	5.25	5.00	18.68	111.53	5.97	118.35	57.70
30	0.67	4.63	6.45	17.28	100.95	5.84	132.70	75.09
30	0.66	5.56	4.20	15.95	86.79	5.44	161.00	94.47
30	0.76	4.89	....	14.68	83.02	5.66	126.40	92.37
30	0.51	....	5.40	14.99	85.74	5.72	142.80	92.27
30	0.45	4.78	6.40	16.35	97.26	5.95	140.40	....
30	0.70	5.09	3.80	12.79	70.30	5.50	117.50	....
403	0.53	4.87	4.35	14.37	82.25	5.72	106.40	64.87
403	0.47	5.00	4.50	14.04	80.97	5.77	95.70	52.53
403	0.49	5.37	3.90	14.10	76.47	5.42	91.50	65.40
403	0.46	4.69	6.50	16.10	91.71	5.70	102.50	68.30
403	0.52	4.93	4.50	14.44	78.74	5.45	104.70	59.90
403	0.64	5.29	5.00	14.58	71.53	4.90	.....	....
403	0.39	4.98	2.30	10.61	53.46	5.04	144.78	....
403	0.47	....	6.00	15.52	91.83	5.92	.....	....
536	0.49	4.31	3.40	12.51	68.37	5.47	.....	....
Av.	0.56	5.06	4.80	15.12	85.50	5.63	121.9	....

The energy value was determined in an Emerson calorimeter equipped with a Daniels adiabatic jacket.[2]

There was no constant increase or decrease in the values for nitrogen, lactose, fat, total phosphorus, inorganic phosphorus or solids-not-fat with the advance of the stage of lactation.

[2] The authors wish to thank Dr. S. Brody, Department of Dairy Husbandry, University of Missouri, for the use of the Emerson calorimeter.

The energy value, total solids, energy accounted for by non-lactose sources, and the number of milligrams of fat per calorie decreased with advanced lactation. The ratio of lactose to fat was very irregular. The number of determinations made was too small to show a definite ratio of phosphorus to lactose (table 1a).

TABLE 1a

The composition of goat milk (calculated relationships)

GOAT NO.	PROTEIN	NITROGEN PER CALORIE	FAT PER CALORIE	LACTOSE PER CALORIE	RATIO LACTOSE TO FAT	PHOSPHORUS TO LACTOSE	ENERGY FROM NON-LACTOSE SOURCE	ENERGY FROM NON-FAT SOURCE	ENERGY FROM SOLIDS NOT-FAT
	%	*mg.*	*mg.*	*mg.*					
348	3.77	5.19	43.45	52.85	1: 0.82	.....	90	69	14.3
348	4.15	6.91	57.62	55.71	1: 1.03	.....	71	45	10.8
348	....	....	46.27	47.09	1: 0.98	1: 44.4	91	65	13.7
30	4.29	6.66	65.94	45.84	1: 1.43	1: 34.8	83	41	10.9
30	4.19	7.57	49.94	64.03	1: 0.78	1: 34.5	65	48	11.7
30	4.85	9.16	....	59.92	.....	1: 38.7	64	..	....
30	3.27	5.98	64.99	....	.....	.....	..	36	9.6
30	2.84	4.58	67.91	49.10	1: 1.38	1: 34.0	79	38	9.9
30	4.46	9.94	55.79	72.52	1: 0.77	1: 43.4	50	35	9.0
403	3.36	6.41	54.58	59.15	1: 0.92	1: 45.7	63	42	10.0
403	2.99	5.79	57.35	61.75	1: 0.92	1: 52.2	61	39	9.5
403	3.13	6.43	52.63	53.66	1: 0.98	1: 58.6	55	40	10.2
403	2.92	4.98	73.15	51.11	1: 1.43	1: 45.7	73	31	9.6
403	3.31	6.58	58.98	62.56	1: 0.94	1: 47.0	59	37	9.9
403	4.08	8.95	72.14	78.89	1: 0.97	.....	52	25	9.6
403	2.52	7.38	44.40	93.22	1: 0.47	1: 34.4	33	32	8.3
403	2.97	5.07	67.43	....	.....	.....	..	36	....
536	3.15	7.21	50.34	63.09	1: 0.80	.....	51	37	9.1
Av.	3.54	6.75	57.82	60.28	1: 0.96	.....	..	..	10.4

The determined energy was compared with the calculated energy, using the values of Malcolm and Hall ('07–'08), fat 9.318, protein 5.860 and lactose 3.950 calories per gram, and also by using their value of 5650 calories per gram for total solids (table 2).

In table 2 it may be seen that the energy value of milk can be estimated with considerable accuracy by the use of protein, lactose and fat percentages and also by the use of the percentage of total solids. These two methods give very nearly

the same value and both are slightly above the determined energy value.

The equation $y = a + bx$ was fitted to the fat percentages and energy values by the method of least squares. The curve of the equation is plotted in figure 1.

TABLE 2

Energy content of goat milk

SAMPLE NO.	BY DETERMINATION CALORIES PER 100 ML.	BY CALCULATION FROM PROTEIN, FAT AND LACTOSE	BY CALCULATION FROM MILK SOLIDS AT 5650 CALS. PER GRAM
1	114.00	90.597	108.028
2	94.04	93.936	91.021
4	100.95	103.527	97.632
5	86.79	85.650	90.117
8	97.26	95.158	92.377
9	70.30	81.689	72.263
10	82.25	79.458	81.190
11	80.97	79.202	79.326
12	76.47	75.893	79.665
13	91.71	96.203	90.965
14	78.74	80.800	81.586
15	71.53	91.394	82.377
16	53.46	55.869	59.946
18	68.37	67.164	70.680
Average	83.34	84.04	84.08

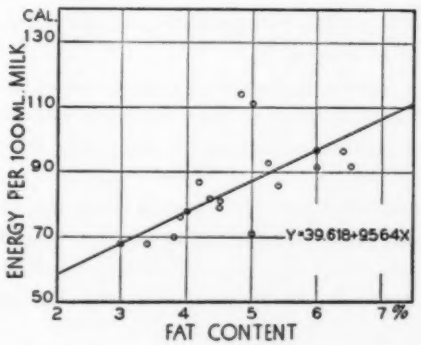

Fig. 1 Graph showing the calories per 100 ml. of milk of increasing fat percentage.

In table 3 comparison is made between the determined energy values and those calculated from the percentage of fat by the equation $y = 39.618 + 9.564x$. The results show that for fat percentages between 3.5 and 5.5 this equation gives the energy value with considerable accuracy.

The same equation was fitted to the total solids and energy values (fig. 2).

A comparison between the determined energy values and the calculated energy values obtained by the use of the equation indicates that the total energy content of goat milk can

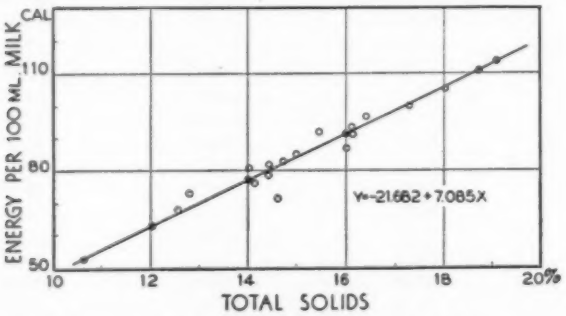

Fig. 2 Graph showing the relation between total solids and energy. This curve permits accurate estimation of energy when the total solids are known.

be estimated with a high degree of accuracy if the percentage of total solids is known.

The results shown in tables 3 and 4 indicate that the percentage of total solids is a more reliable determination than the percentage of fat on which to calculate the energy value of goat milk.

Overman and Gaines ('33) found that the protein-energy ratio increased with an increase of fat percentage in the Holstein breed, while the Ayrshire breed showed a marked decrease in protein-energy ratio as the fat percentage increased.

TABLE 3

Relation of fat to energy content of goat milk

FAT	CALORIES PER 100 ML. DETERMINED	FAT	CALORIES PER 100 ML. CALCULATED [1]	DIFFERENCE
%		%		%
2.3	53.464	2.0	58.746	
3.4	68.368	2.5	63.528	
3.8	70.301	3.0	68.310	
3.9	76.468	3.5	73.092	+7
4.2	86.791	4.0	77.874	+2
4.35	82.255	4.5	82.656	+5
4.5	80.973	5.0	87.438	−21, +22
4.5	78.741	5.5	92.220	+7
4.8	113.998	6.0	97.002	+5
5.0	111.531	6.5	101.784	+11
5.0	71.531			
5.25	94.034			
5.4	85.744			
6.0	91.827			
6.4	97.262			
6.45	100.948			
6.5	91.708			

[1] $Y = 39.618 + 9.564x$.

TABLE 4

Relation of total solids to energy content of goat milk

TOTAL SOLIDS	ENERGY IN CALORIES DETERMINED PER 100 ML.	TOTAL SOLIDS	ENERGY VALUE CALCULATED PER 100 ML.[1]
%		%	
10.61	53.464	10.00	49.168
12.50	68.368	10.50	52.710
12.78	70.301	11.00	56.253
14.04	80.973	11.50	59.795
14.10	76.468	12.00	63.338
14.37	82.255	12.50	66.880
14.43	78.741	13.00	70.423
14.59	71.531	13.50	73.965
14.68	83.019	14.00	77.503
14.99	85.744	14.50	81.050
15.52	91.827	15.00	84.593
15.95	86.791	15.50	88.135
16.10	91.708	16.00	91.678
16.11	94.034	16.50	95.220
16.35	97.262	17.00	98.763
17.28	100.948	17.50	102.305
18.68	111.531	18.00	105.848
19.12	113.998	18.50	109.390
		19.00	112.933

[1] $y = 21.682 + 7.085x$.

In figure 3 the milligrams of nitrogen per calorie were plotted against fat percentage. The calculated equation shows that there is a definite decrease in the nitrogen-energy ratio with increase in fat percentage.

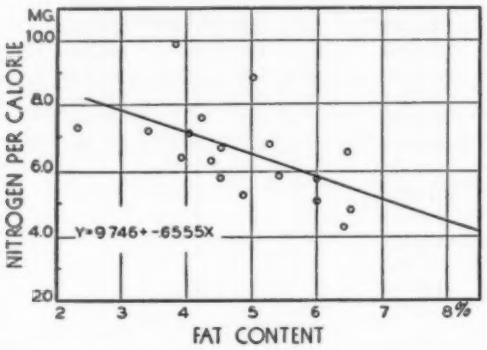

Fig. 3 Showing the definite decrease in protein-energy ratio with increase in fat percentage.

TABLE 5

Comparison of energy values of goat and cow milk

FAT (GOAT)	CALORIES PER POUND BASED ON EQUATION $y = 39.618 + 9.564x$ [1]	FAT (COW)	CALORIES PER POUND BASED ON EQUATION $y = 51.6M\ (2.66 + f)$ [2]
%		%	
3.0	300.24	3.0	292.06
3.5	321.26	3.5	317.85
4.0	342.28	4.0	343.66
4.5	363.30	4.5	369.46
5.0	384.32	5.0	395.26
5.5	405.33	5.5	421.06
6.0	426.35	6.0	446.85
6.5	447.37	6.5	472.66

[1] The equation $y = 39.618 + 9.564x$ gives the result in Calories per 100 ml. These results were converted into the corresponding value per pound (X4.3953).
[2] Gaines, 1928.

In table 5 comparison is made of the energy content of cow's milk with that of goat's milk containing the same fat percentage. Goat's milk containing from 3 to 5% compares favorably in energy content with cow's milk.

SUMMARY

The energy value, fat, nitrogen, lactose, inorganic and total phosphorus, and total solids were determined on eighteen samples of goat milk collected in various stages of lactation.

Comparisons were made between the determined energy value and that obtained by calculating from some constituents. It was found that the energy value could be estimated with considerable accuracy from either the protein, lactose and fat percentages or the percentage of total solids.

The results show that for fat percentages between 3.5 and 5.5 the equation $y = 39.618 + 9.564x$ gives energy values with considerable accuracy.

If the percentage of total solids is known the equation $y = 21.682 + 7.085x$ gives energy values nearly identical with the determined values.

One pound of 4% goat's milk was found to have an average energy value of 342.28 calories in comparison to 343.66 calories for cow's milk.

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